U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371 AND 37 CFR 1.491

ATTORNEY DOCKET NO.: 216180

US APPLICATION NO: Unassigned 10/088966

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/08813	08 SEPTEMBER 2000 (08.09.00)	24 SEPTEMBER 1999 (24.09.99)

TITLE OF INVENTION
NUCLEIC ACID MOLECULES FOR THE DETECTION OF BACTERIA AND PHYLOGENETIC UNITS OF BACTERIA

APPLICANT(S) FOR DO/EO/US
GRABOWSKI, Reiner; BERGHOF, Kornelia

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 USC 371 and 37 CFR 1.491.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371 and 37 CFR 1.491.

3. ☒ This is an express request to begin national examination procedures (35 USC 371(f)).

4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).

5. ☒ A copy of the International Application as filed (35 USC 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ An English language translation of the International Application as filed (35 USC 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 USC 371(c)(4)).

10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 USC 371(c)(5)).

11. Nucleotide and/or Amino Acid Sequence Submission
    a. ☐ Computer Readable Form (CRF)
    b. Specification Sequence Listing on:
       i. ☐ CD-ROM or CD-R (2 copies); or
       ii. ☒ Paper Copy
    c. ☐ Statement verifying identity of above copies

Items 12 to 19 below concern other document(s) or information included:

12. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
    ☒ Form PTO-1449
    ☒ Copies of Listed Documents

13. ☐ An assignment for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

14. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

15. ☐ A substitute specification.

16. ☐ A change of power of attorney and/or address letter.

17. ☒ Application Data Sheet Under 37 CFR 1.76

18. ☒ Return Receipt Postcard

19. ☒ Other items or information: Amendments to Specification and Claims Made Via Preliminary Amendment; Pending Claims After Entry of Preliminary Amendment; Copy of International Search Report

U.S. APPLICATION NO	INTERNATIONAL APPLICATION NO	ATTORNEY DOCKET NO
Unassigned 10/088966	PCT/EP00/08813	216180

20. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,040.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 890.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO, but international search fee (37 CFR 1.445(a)(2))paid to USPTO ..... $ 740.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... $ 710.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1) to (4) ..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT=	$890.00	
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	21 -20=	1	x $ 18.00	$18.00	
Independent Claims	1 - 3 =	0	x $ 84.00	$0.00	
☐ Multiple Dependent Claim(s) (if applicable)			+$280.00	$	
TOTAL OF ABOVE CALCULATIONS=				$908.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL=				$908.00	
Processing fee of **$130.00** for furnishing English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date.				$	
TOTAL NATIONAL FEE=				$908.00	
Fee for recording the enclosed assignment. The assignment must be accompanied by an appropriate cover sheet. **$40.00** per property +				$	
TOTAL FEE ENCLOSED=				$	
				Amount to be refunded	$
				charged	$

a. ☒ A check in the amount of $908.00 to cover the above fee is enclosed.

b. ☐ Please charge Deposit Account No. 12-1216 in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 12-1216. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Customer Number: 23460

23460

PATENT TRADEMARK OFFICE

Carol Lardner, Registration No. 35,243
One of the Attorneys for Applicant(s)

Date: March 22, 2002

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NO
Unassigned 10/088966	PCT/EP00/08813	216180

CERTIFICATION UNDER 37 CFR 1.10

"Express Mail" Label Number: EL643546523US

Date of Deposit: March 22, 2002

I hereby certify that this express request to begin national examination procedures under 35 USC 371(f) of the International Patent Application referenced above, including all of the items listed thereon as enclosures, is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Commissioner for Patents, Attention: DO/EO/US, Washington, D.C. 20231.

Irina Mikitiouk

Printed Name of Person Signing:

I. Mikitiouk

Signature

PATENT
Attorney Docket No. 216180

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Grabowski et al.

Art Unit: Unassigned

Application No. Unassigned
(U.S. National Phase of PCT/EP00/08813)

Examiner: Unassigned

Filed: March 22, 2002

For: NUCLEIC ACID MOLECULES FOR THE DETECTION OF BACTERIA AND PHYLOGENETIC UNITS OF BACTERIA

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks.

AMENDMENTS

IN THE SPECIFICATION:

Replace the paragraph beginning at page 20, line 4, with:

- Wash buffer 2

100 mM Tris Gibco, No. 15504-038)	12.15 g
150 mM NaCl (Merck, No. 6404.5000)	8.78 g
0.05% Tween 20 (Serva, No. 37470)	0.5 g
0.5% blocking reagent (Boehringer)	Dissolve 5 g in D1 (see below) at 60 °C

10 µg/ml herring sperm
Dilute to 1 liter with double-distilled water and adjust to pH 7.5

Replace the paragraph beginning at page 21, line 23, with:

ELISA procedure:

200 µl binding buffer and 1 µl probe are applied for each well. The microtiter plate is covered with an adhesive film and left to stand for two hours at room temperature. The PCR amplificates to be examined are thawed at room temperature, mixed with the denaturation bufffer in the ratio of 1:1, and incubated for 10 minutes at room temperature. Then 10 µl of this probe is placed into the wells, which have been emptied in the meantime. In addition, 100 µl hybridization buffer is added to each well and incubated for 30 minutes at 37 – 60 °C. To wash, the wells are emptied, filled with 200 µl wash buffer 1 which has been preheated to 37 – 60 °C, and incubated for 2 minutes at the same temperature. This washing step is done three times.

Replace the paragraph beginning at page 22, line 1, with:
After the wash buffer has been carefully removed, the Anti-Dig-POD-antibody (DAKO) is diluted 1:3000 (1 µl in 3 ml wash buffer 2), and 100 µl of this solution is placed into each of the dry wells. This arrangement is incubated in the incubator at 37 °C for 30 minutes.

Replace the paragraph beginning at page 22, line 6, with:
Then the microtiter plate is washed three times with 200 µl wash buffer 2 per depression. Then 100 µl of the BM Blue dye (Boehringer) is added per well. After 15 minutes the reaction is stopped by addition of 100 µl 0.5 M H_2SO_4. The absorbance of the samples is measured in the ELISA reader.

IN THE CLAIMS:

Please cancel claims 1-74.

Please add the following new claims:

75. (New) Nucleic acid molecules as a probe and/or a primer for detection of bacteria, selected from:

a) nucleic acid molecules comprising at least one sequence with any of the SEQ ID NOs: 1 to 530 and/or a sequence from position 2667 to 2720, 2727 to 2776, 2777 to 2801, 2801 to 2832, 2857 to 2896, 2907 to 2931, 2983 to 2999 and/or 3000 to 3032 according to SEQ ID NO: 1; or nucleic acids which are homologous, analogous, or at least 70% identical with them;
b) nucleic acid molecules which hybridize specifically with a nucleic acid according to a);

c) nucleic acid molecules which exhibit 70% identity with a nucleic acid according to a) or b); and
d) nucleic acid molecules which are complementary to a nucleic acid according to any of a) to c).

76. (New) Nucleic acid molecule according to Claim 75, characterized in that the nucleic acid molecule according to alternative a) exhibits a sequence selected from SEQ ID NO: 211 and SEQ ID NO: 212.

77. (New) Nucleic acid molecule according to Claim 75, characterized in that the nucleic acid molecule according to alternative c) exhibits at least 90% identity with a nucleic acid according to a) or b).

78. (New) Nucleic acid molecule according to Claim 75, characterized in that it is at least 10 nucleotides long.

79. (New) Nucleic acid molecule according to Claim 78, characterized in that it is at least 14 nucleotides long.

80. (New) Nucleic acid molecule according to Claim 75, characterized in that the nucleic acid molecule is modified such that up to 20% of the nucleotides in 10 successive nucleotides are replaced by nucleotides which do not occur naturally in bacteria.

81. (New) Nucleic acid molecule according to Claim 75, characterized in that the nucleic acid molecule is modified or labeled so that it can generate a signal in analytical detection procedures which are known per se, with the modification selected from (i) radioactive groups, (ii) colored groups, (iii) fluorescent groups, (iv) groups for immobilization of a solid phase, and (v) groups which allow a direct or indirect reaction, especially using antibodies, antigens, enzymes, and/or substances with affinity to enzymes or enzyme complexes.

82. (New) Combination of at least 2 nucleic acid molecules, selected from
a) a combination of at least one DNA molecule which is shortened in comparison with the sequence SEQ ID NO: 1, position 2571 to 2906, and at least one DNA molecule which is shortened or not shortened in comparison with the transcribed spacer between the 23 S and 5 S genes corresponding to

position 2907 to 2999 in SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

b) a combination of at least one DNA molecule which is shortened or not shortened in comparison with the transcribed spacer between the 23 S and 5 S genes, position 2907 to 2999 of SEQ ID NO: 1, and at least one DNA molecule which is shortened in comparison with the 5 S rDNA gene with the sequence between positions 3000 to 3112 of SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

c) a combination of at least one DNA molecule which is shortened or not shortened in comparison with the 23 S gene with the sequence from position 2907 to 2999 of SEQ ID NO: 1, and at least one shortened DNA molecule from the 5 S rDNA gene from position 3000 to 3112 of SEQ ID NO: 1, or DNA molecules with are homologous, analogous, or at least 75% identical with them;

d) a combination of at least one DNA molecule which is shortened in comparison with the 23 S gene with the sequence from position 2571 to 2906 of the SEQ ID NO: 1 and at least one shortened DNA molecule from the 5 S rDNA gene from position 3000 to 3112 of SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

e) a combination of 2 nucleic acid molecules according to Claim 75; and

f) a combination containing at least one DNA molecule which hybridizes with a region hybridizing at least 100 nucleotides upstream from the 3' end of the 23 S rDNA, therefore within the 23 S rDNA;

wherein the combination according to any of a) to f) can also be a combined DNA molecule comprising at least 15 base pairs, for detection of bacteria or phylogenetic groups of bacteria.

83. (New) Combination of at least 2 nucleic acid molecules of Claim 82, wherein the bacteria are enterobacteria.

84. (New) Combination according to Claim 82, characterized in that it contains at least one nucleic acid molecule according to alternative a) that exhibits a sequence selected from SEQ ID NO: 211 and SEQ ID NO: 212.

85. (New) Combination according to Claim 84, characterized in that it contains a nucleic acid molecule with a sequence according to SEQ ID NO: 211 and a nucleic acid molecule with a sequence according to SEQ ID NO: 212.

86. (New) Method for detecting bacteria in an analytical sample, comprising the step of bringing the analytical sample into contact with a nucleic acid or a combination of nucleic acids according to Claim 75, and detecting suitable hybrid nucleic acids comprising the added nucleic acid and bacterial nucleic acid.

87. (New) Method for detecting bacteria in an analytical sample of Claim 86, wherein the bacteria are enterobacteria.

88. (New) Method according to Claim 86, characterized in that the process involves a PCR amplification of the nucleic acid to be detected.

89. (New) Method according to Claim 86, characterized in that the process involves a Southern Blot hybridization.

90. (New) Method for detecting bacteria in an analytical sample, comprising the step of bringing the analytical sample into contact with a nucleic acid or a combination of nucleic acids according to Claim 82, and detecting suitable hybrid nucleic acids comprising the added nucleic acid and bacterial nucleic acid.

91. (New) Method for detecting bacteria in an analytical sample according to Claim 90, wherein the bacteria are enterobacteria.

92. (New) Method for amplifying bacterial DNA of a multiplicity of different taxonomic units, especially genera and species, using primers according to Claim 75, in which in a first amplification step the DNA for high taxonomic units such as classes, phyla or families is amplified with conserved primers, and, optionally, in at least one further amplification step (EN) parts of the first amplification fragment which are specific for genera or species can be multiplied with nested, increasingly variable primers, and, optionally, in a further step, the DNA fragments obtained by amplification which are specific for genera or species are detected by means of probes.

93. (New) Method according to Claim 92, characterized in that the process involves a PCR amplification of the nucleic acid to be detected.

94. (New) Method according to Claim 92, characterized in that the process involves a Southern Blot hybridization.

95. (New) Method for amplifying bacterial DNA of a multiplicity of different taxonomic units, especially genera and species, using primers according to Claim 82, in which in a first amplification step the DNA for high taxonomic units such as classes, phyla or families is amplified with conserved primers, and, optionally, in at least one further amplification step (EN) parts of the first amplification fragment which are specific for genera or species can be multiplied with nested, increasingly variable primers, and, optionally, in a further step, the DNA fragments obtained by amplification which are specific for genera or species are detected by means of probes.

REMARKS

The present application is the U.S. national phase of a PCT application. The specification has been amended to correct inadvertent typographical and translation errors. In addition, claims 1-74 have been cancelled, and claims 75-95 have been added. The claims have been amended to conform the claims to U.S. patent practice and to eliminate multiple claim dependencies. Applicants reserve the right to reinstate canceled claims. No new matter has been added by way of these amendments.

The application is considered to be in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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Date: March 22, 2002

PATENT
Attorney Docket No. 216180

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Grabowski et al.

Art Unit: Unassigned

Application No. Unassigned
(U.S. National Phase of PCT/EP00/08813)

Examiner: Unassigned

Filed: March 22, 2002

For: NUCLEIC ACID MOLECULES FOR THE DETECTION OF BACTERIA AND PHYLOGENETIC UNITS OF BACTERIA

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

75. Nucleic acid molecules as a probe and/or a primer for detection of bacteria, selected from:

a) nucleic acid molecules comprising at least one sequence with any of the SEQ ID NOs: 1 to 530 and/or a sequence from position 2667 to 2720, 2727 to 2776, 2777 to 2801, 2801 to 2832, 2857 to 2896, 2907 to 2931, 2983 to 2999 and/or 3000 to 3032 according to SEQ ID NO: 1; or nucleic acids which are homologous, analogous, or at least 70% identical with them;

b) nucleic acid molecules which hybridize specifically with a nucleic acid according to a);

c) nucleic acid molecules which exhibit 70% identity with a nucleic acid according to a) or b); and

d) nucleic acid molecules which are complementary to a nucleic acid according to any of a) to c).

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77. Nucleic acid molecule according to Claim 75, characterized in that the nucleic acid molecule according to alternative c) exhibits at least 90% identity with a nucleic acid according to a) or b).

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81. Nucleic acid molecule according to Claim 75, characterized in that the nucleic acid molecule is modified or labeled so that it can generate a signal in analytical detection procedures which are known per se, with the modification selected from (i) radioactive groups, (ii) colored groups, (iii) fluorescent groups, (iv) groups for immobilization of a solid phase, and (v) groups which allow a direct or indirect reaction, especially using antibodies, antigens, enzymes, and/or substances with affinity to enzymes or enzyme complexes.

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b) a combination of at least one DNA molecule which is shortened or not shortened in comparison with the transcribed spacer between the 23 S and 5 S genes, position 2907 to 2999 of SEQ ID NO: 1, and at least one DNA molecule which is shortened in comparison with the 5 S rDNA gene with the sequence between positions 3000 to 3112 of SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

c) a combination of at least one DNA molecule which is shortened or not shortened in comparison with the 23 S gene with the sequence from position 2907 to 2999 of SEQ ID NO: 1, and at least one shortened DNA molecule

from the 5 S rDNA gene from position 3000 to 3112 of SEQ ID NO: 1, or DNA molecules with are homologous, analogous, or at least 75% identical with them;

d) a combination of at least one DNA molecule which is shortened in comparison with the 23 S gene with the sequence from position 2571 to 2906 of the SEQ ID NO: 1 and at least one shortened DNA molecule from the 5 S rDNA gene from position 3000 to 3112 of SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

e) a combination of 2 nucleic acid molecules according to Claim 75; and

f) a combination containing at least one DNA molecule which hybridizes with a region hybridizing at least 100 nucleotides upstream from the 3' end of the 23 S rDNA, therefore within the 23 S rDNA;

wherein the combination according to any of a) to f) can also be a combined DNA molecule comprising at least 15 base pairs, for detection of bacteria or phylogenetic groups of bacteria.

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88. Method according to Claim 86, characterized in that the process involves a PCR amplification of the nucleic acid to be detected.

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91. Method for detecting bacteria in an analytical sample according to Claim 90, wherein the bacteria are enterobacteria.

92. Method for amplifying bacterial DNA of a multiplicity of different taxonomic units, especially genera and species, using primers according to Claim 75, in which in a first amplification step the DNA for high taxonomic units such as classes, phyla or families is amplified with conserved primers, and, optionally, in at least one further amplification step (EN) parts of the first amplification fragment which are specific for genera or species can be multiplied with nested, increasingly variable primers, and, optionally, in a further step, the DNA fragments obtained by amplification which are specific for genera or species are detected by means of probes.

93. Method according to Claim 92, characterized in that the process involves a PCR amplification of the nucleic acid to be detected.

94. Method according to Claim 92, characterized in that the process involves a Southern Blot hybridization.

95. Method for amplifying bacterial DNA of a multiplicity of different taxonomic units, especially genera and species, using primers according to Claim 82, in which in a first amplification step the DNA for high taxonomic units such as classes, phyla or families is amplified with conserved primers, and, optionally, in at least one further amplification step (EN) parts of the first amplification fragment which are specific for genera or species can be multiplied with nested, increasingly variable primers, and,

optionally, in a further step, the DNA fragments obtained by amplification which are specific for genera or species are detected by means of probes.

8/pdt

Nucleic acid molecules for the detection of bacteria and phylogenetic units of bacteria

The present invention relates to nucleic acid molecules which allow the identification of bacteria or groups of bacteria.

Bacteria are an ubiquitous component of the human environment. But they cause problems so frequently, as agents of food spoilage or pathogens, that effective, rapid, and reliable diagnosis is of great importance.

The most important microorganisms which cause food spoilage are Clostridium botulinum, the cause of botulism; Campylobacter jejuni; Clostridium perfringens; Cryptosporidium parvum, enteropathogenic strains of Escherichia coli; Shigella; Listeria monocytogenes; Salmonella species; Staphylococcus aureus; Vibrio vulnificus; and Yersinia enterolytica. The General Accounting Office (GAO) reported in 1996 that from 6.5 to 81 million cases of food poisoning occur in the USA every year. The US Food and Drug Administration (FDA) estimates that 2 – 3% of all food poisonings lead to chronic secondary diseases. It is also estimated that 2 - 4 million cases of sickness in the US are caused by more than 2000 strains of Salmonella. Those horrifying statistics could be extended to other food spoilage organisms. Food poisonings do not just cause human suffering, though, with death in extreme cases, but also substantial economic damage, which is estimated at 5.6 – 9.4 billion dollars for the US in 1991, for instance.

It is generally known that microorganisms, as agents of infection, present great danger. Their potential can hardly be estimated. For instance, the World Health Report from the WHO indicates statistical orders of magnitude. In 1998, for instance, pathogens, including parasites, were responsible for 9.8 million deaths (not counting prenatal or postnatal infections). That amounts to 18.2% of all deaths due to disease. The dangerous pathogens cannot be summarized as well as the food spoilage organisms, as they are recruited from many phylogenetic branches of the Eubacteria. There is a particularly great "infectious potential" in the Enterobacteria family, in particular.

In combating bacteria pathogenic for humans, identification of the microbes causing a disease or a pathologic symptom is a significant step. Often the proper medical

measures can be applied only after the identification. Furthermore, detection methods for bacteria which work well could also be used as preventive tools in food quality assurance.

Classical detection of bacteria consists of microbiological identification, which usually involves isolation on selective media containing agar. This procedure has two significant disadvantages, however. First, the detection is often not reliable or specific. Second, many bacteria require a growth period of at least 18 hours for isolation as colonies. In many cases, a secondary isolation or a secondary detection are also necessary. Everything considered, diagnosis times up to a week are not unusual. In addition to that, there are also pathogenic microbes which cannot be cultured (J. J. Byrd et al., 1991, Appl. Environ. Microbiol. 57, 875-878). In a time of rapid means of transport and global trade in goods, though, rapid diagnostic methods which in the optimal case should not take longer than 24 hours, are essential to prevent the spread of pathogens or world-wide food poisonings from just a single local source.

Various procedures have been developed in recent years to meet modern requirements. They are intended to provide rapid and reliable routine identification of microbes. For example, immunologic methods utilize the specific binding of monoclonal or polyclonal antibodies to bacterial surface antigens. Such procedures are used particularly for serotyping for Salmonella, for instance. In general, to be sure, detection by ELISA is relatively rapid, but it requires processing and isolation of the specific antigens, and that can have many problems. Bacterial detection methods utilizing DNA probes have proven to be particularly capable because they are very sensitive, relatively specific, and can be used to detect microorganisms in a total experimental period of 2 – 3 days.

Background of the invention

The invention consists in providing specific DNA sequences and selecting DNA regions which are particularly suitable for detecting bacteria. Thus this application is based on the identification of organisms by their genetic information. Using deviations of as little as a single component in the nucleotide sequence in certain DNA regions it is already possible to differentiate species.

Historically considered, ribosomal RNA genes have already been used for phylogenetic classification of organisms. Comparisons of sequences of the 5 S and 16 S ribosomal genes in different bacteria have led to significant corrections in assignments of relatedness and to discovery of the kingdom of the Archaebacteria. Because of its size and the corresponding high sequencing effort, 23 S RNA has only in recent years been used for systematic classifications.

Direct sequencing of genes of microorganisms to be identified was too expensive and time-consuming in practical use. In the 1980s, therefore, specific nucleotide probes were used to detect bacteria. While those can show very good specificity, the detection limit is often too low. The probe technology was substantially improved by combination with amplification techniques, which reproduce the nucleotide sequence to be detected and thus substantially increase the sensitivity of detection. In an extreme case, it is possible to detect a single isolated genome. In practice, losses occur in isolation of DNA, increasing the detection limit to about 10^2 to 10^4 cells.

On the basis of fundamental research, DNA probes from the 5 S, 16 S and 23 S genes were utilized for practical applications. For instance, one should note these patents: Nietupski et al. (US 5,147,778) for detection of Salmonella; Mann and Wood (US 6,554,144) for detection of Yersinia species; Leong (EP 04 79 117 A1) for detection of various Gram negative and Gram positive bacteria; Carico et al. (EP 1 33 671 B1) for detection of various enterobacterial species; Shah et al. (EP 03 39 783 B1) for detection of Yersinia enterolytica; Carrico (EP 01 63 220 B1) for detection of Escherichia coli; Hogan et al. (WO 88/03957) for detection of species of Enterobacteria, Mycobacterium, Mycoplasma and Legionella; Leiser et al. (WO 97/41253) for detection of various microorganisms; Grosz and Jensen (WO 95/33854) for detection of Salmonella enterica; Stackebrandt and Curiaie (EP 03 14 294 A2) for detection of Listeria monocytogenes; Wolff et al. (EP 04 08 077 A2), Hogan and Hammond (US 5,681,698) for detection of Mycobacterium kansasii; Hogan et al. (US 5,679,520) for detection of various bacteria; Kohne (US 5,567,587) particularly for detection of bacterial RNA; Kohne (US 5,714,324) for detection of various bacteria; Pelletier (WO 94/28174) for detection of Legionella; and Kohne (US 5,601,984) for detection of various bacteria. Most of the patents relate to the sequence of the 16 S rDNA gene, and many also relate to the 23 S rDNA.

It appeared, though, that the latter genes are not suitable for many differentiation operations in practical use because they are too strongly conserved. Closely related microorganisms in particular cannot be differentiated. On the other hand, the 5 S rDNA gene is generally too variable and its differentiation potential is too low for practical use, even though it was initially used for phylogenetic studies in basic research because of its small size.

As the 5 S, 16 S and 23 S rDNA genes have many disadvantages as diagnostic aids, DNA regions which could be used for identification of all eubacteria were sought. Such a DNA region should have very variable and, at the same time, strongly conserved sequences. Then the variable regions would be useful to differentiate closely related species, such as strains and species. The conserved sequences would be used to detect more distantly related bacteria or higher taxonomic units.

In the very recent past, the 16 S – 23 S transcribed spacer has been discussed in the literature in the context of extensive studies on ribosomal operons. Their applicability in systematic bacteriology has been questioned, though. For example, Nagpal et al. (J. Microbiol. Meth. 33, 1998, p. 212) considered the utility of these spacers very critically: A major problem with this transcribed rDNA spacer is that it frequently contains tRNA insertions. Such insertions represent dramatic changes in the sequences, and do not necessarily have a relation to phylogenetic separations. However, they have been used in the past to utilize the length polymorphism which they cause as a phylogenetic characteristic (Jensen et al. 1993, Appl. Envir. Microb. 59, 945-952; Jensen, WO 93/11264; Kur et al. 1995, Acta Microb. Pol. 44, 111-117).

The transcribed spacer between the 23 S and 5 S rDNA is an alternative target sequence for identification of bacteria. For instance, Zhu et al. (J. Appl. Bacteriol. 80, 1996, 244-251) published detection of Salmonella typhi using this diagnostic DNA region. However, the general utility of this spacer for detecting other bacteria cannot be derived from that work. There are very many examples which indicate that a DNA region is suitable only for identifying one or a few species of bacteria. Individual patents imply a potential but very limited applicability of the 23 S – 5 S transcribed DNA region for bacterial diagnosis. Those all have in common that their applicability is limited to just a single bacterial species, specifically, to detection of Legionella

(Heidrich et al., EP 07 39 988 A1), Pseudomonas aeruginosa (Berghof et al., DE 197 39 611 A1) and Staphylococcus aureus (Berghof et al., WO 99/05159).

The technical problem underlying the present invention consists in providing materials and processes which allow to detect any desired bacterium (preferably from the Enterobacteria group) in a material being examined.

This problem is solved according to the invention by a nucleic acid molecule as a probe and/or a primer for detection of bacteria, selected from

a) a nucleic acid comprising at least one sequence with any of the SEQ ID NOs: 1 to 530 and/or a sequence from position 2667 to 2720, 2727 to 2776, 2777 to 2801, 2801 to 2832, 2857 to 2896, 2907 to 2931, 2983 to 2999, and/or 3000 to 3032 according to SEQ ID NO: 1; or nucleic acids homologous with them;

b) a nucleic acid which hybridizes specifically with a nucleic acid according to a);

c) a nucleic acid which exhibits 70%, and preferably at least 90%, identity with a nucleic acid according to a) or b);

d) a nucleic acid which is complementary to a nucleic acid according to any of a) to c);

and/or

combinations of the nucleic acids according to any of a) to d), except for the SEQ ID NO:1.

Further claims concern preferred embodiments.

In one particularly preferred embodiment, the presence of Enterobacteria in a sample being analyzed is shown by the analysis sample being brought into contact with a probe which detects the presence of a nucleic acid from the 23 S/5 S rDNA genome segment of the Enterobacteria.

The sequence specified as NO: 1 in Claim 1 is derived from *E. coli*. Homologous DNA sequences are those derived from bacteria other than the *E. coli* sequence shown,

but in which the genome segment from the other bacteria corresponds to the sequence based on SEQ ID NO:1. For more details, we refer to the definition of homologous DNA sequences, below.

The nucleic acid molecule according to the invention comprises preferably at least 10 nucleotides, and especially preferably at least 14 nucleotides. Nucleic acid molecules of these lengths are used preferably as primers, while nucleic acids used as probes preferably comprise at least 50 nucleotides.

In another preferred embodiment, nucleotides of the probe or the primer can be replaced by modified nucleotides containing, for instance, attached groups which ultimately are used for a detection reaction. Particularly preferred derivatizations are specified in Claim 4.

In another preferred embodiment, combinations of the specified nucleic acid molecules are used. Selecting the particular combination of nucleic acid molecules allows adjustment of the selectivity of the detection reaction. In doing so, selection of the primer combinations and/or probe combinations can establish the conditions of the detection reactions so that they either demonstrate generally the presence of bacteria in a sample, or specifically indicate the presence of a certain bacterial species.

A kit according to the invention contains at least one nucleic acid according to the invention together with the other usual reagents used for nucleic acid detection. They include, among others, suitable buffers and detection agents such as enzymes with which, for example, biotinylated nucleic acid hybrids which are formed can be detected.

In another preferred embodiment, called Consensus PCR here, the process is carried out according to Claim 8. First, a nucleic acid fragment is amplified by use of conserved primers (those hybridize to nucleic acids of different bacterial taxonomic units). Then more specific nucleic acid segments are detected by use of other more specific nucleic acids (these hybridize with only a few taxonomic units or only with a certain species). The latter allow then a conclusion about the presence of a particular genus, type or species in the sample being analyzed.

Various established detection procedures can be employed to detect nucleic acids in the process used. They include Southern Blot techniques, PCR techniques, LCR techniques, etc.

In one broad study, transcribed spacer between 23 S and 5 S rDNA was examined for its general usefulness as a diagnostic target molecule. For this purpose, genomic DNA from very many bacterial strains was isolated, purified, cloned into a vector, sequenced, and finally evaluated in an extensive sequence comparison. Surprisingly, this sequence segment was suitable for identification of almost all bacterial species. With the encouragement of that finding, the analyses were extended to the adjacent regions of the spacer. All in all, DNA fragments from all bacterial classes or smaller phylogenetic units were examined. They have lengths of 400 – 750 base pairs and include the end, i. e., the last 330 – 430 nucleotides (depending on the species) of the 23 S rDNA gene, the transcribed spacer, and the complete 5 S rDNA gene. The total size of the fragments is 400 – 750 base pairs. The experiments showed that the 23 S rDNA gene and the 5 S rDNA gene are adjacent in almost all bacterial species. This information is an important prerequisite for use and applicability of this invention.

This invention is particularly based on the fact that a DNA region which can contain significant portions of at least two adjacent genes is selected for detection of microorganisms. In practice, the usefulness of the region is determined particularly by its phylogenetic variablility. There can be quite contrary requirements, depending on whether distantly related bacteria, taxonomic units, or strains of a species are to be detected. Now the frequency of occurrence of both variable and conserved regions is greater for two genes than for one, as the example of the 23 S – 5 S tandem shows. Thus the use of two adjacent genes, including the variable intercalated sequences is a substantial advantage.

It was also found that the end of the 23 S rDNA gene, the 5 S rDNA gene, and the transcribed spacer between them contain nucleotide sequences which cover a wide range from very variable to very conserved. A fine analysis of this region provided further very interesting conclusions about the differentiation potential of various phylogenetic bacterial units (Figure 2, Table 6). Nearly all taxonomic units can be detected and/or differentiated by using subregions. More or less variable regions are

shown in Figure 2 with the sections 1 – 9, while the strongly conserved regions are intercalated between and adjacent to them. The latter are thus particularly suitable for detecting higher taxonomic units, such as the whole Eubacteria or classes or divisions of them.

The phylogenetic dendrogram in Figure 1 provides another indication of the usefulness of the region. It can be seen that the 23 S rDNA – 5 S rDNA region allows very good differentiation with respect to coarse classification, as members of the Proteobacteria are assigned to 1 – 2 groups, while the Firmicutes are separated. Furthermore, the lengths of the branches, even for closely related species, indicates that they can be distinguished well from each other. Here a phylogenetically correct assignment of close relatives in the dendrogram is quite undesirable, because then they would lie in a closely connected coherent group and perhaps could not be distinguished as easily from one another.

Detailed description of the figures

Figure 1: Phylogenetic dendrogram of some bacteria detected in this work. It can be seen that the Proteobacteria and the Firmicutes form branches which can be separated.

Figure 2: Schematic representation of the ribosomal region described herein comprising the terminal region of the 23 S rDNA, the transcribed spacer, and the 5 S rDNA. This region, or parts of it, is used to detect bacteria. Table 6 shows a detailed characterization of individual domains.

Figures 3-7: Detection of enterobacteria by PCR. The figures show gels stained with ethidium bromide. The presence of bands is characteristic of the presence of Enterobacteria. The upper halves of the figures show positive findings, while the lower halves show the negative controls. Table 7 summarizes the use of the primer. A mixture of Bgl 1 and Hinf 1 of restriction-digested BR328 plasmid DNA (Boehringer Mannheim) was used as the DNA size standard. The DNA size markers include the restriction fragment sizes 154, 220, 234, 298, 394, 453, 517, 653, 1033, 1230, 1766 and 2176 base pairs.

Figure 8: Plan of a consensus PCR. Conserved primers are arranged peripherally, and less-conserved primers are nested internally. In a first step, consensus PCR

allows amplification of DNA with high taxonomic breadth, in the extreme case of all bacterial species. In the subsequent steps, there can be further rounds of amplification. They may be performed in separate vessels, with primers specific for smaller taxonomic units. In the final step, probes can be used which likewise contribute to the specificity of the detection and which can also aid observation of the detection, such as with dyes. Here, and in this figure, the following nomenclature is used: Primer A: the most conserved primers, and the ones with the most peripheral positions in the detection system; Primer [A, B, C ...]: the sequence of primers in the nesting as shown above; Primer [capital letter]1: forward primer; Primer [capital letter]2: reverse primer; Primer [capital letter][number][lower-case letter]: the lower-case letters characterize similar primers, or primers which hybridize at homologous or comparable positions within a target DNA. The probe is preferably in the central, highly variable, region if species or strains are to be detected.

Example 1): Detection of the Enterobacteriaceae family

Genomic DNA was isolated, using standard procedures which are themselves known, from pure cultures of the bacteria listed in Table 1. Quantities of about 1 to 100 ng from each of these preparations were used in PCRs. The reaction solution had the following composition:

genomic DNA	1 µl
H_2O	19.8 µl
Buffer (10x)[*1]	2.5 µl
dNTP (10 mM)[*2]	0.25 µl
forward primer (10 µM)[*3]	0.20 µl
reverse primer (10 µM)[*3]	0.20 µl
$MgCl_2$	0.75 µl
Taq polymerase (5 U/µl)[*1]	0.3 µl

[*1]: Buffer and enzyme from Biomaster or any other source.

[*2]: Nucleotides from Boehringer Mannheim or any other source.

[*3]: Equimolar quantities of primers.
In the case of mixtures, each forward and reverse primer has a total final concentration of 10 µM.

The PCR was done in a Perkin Elmer 9600 Thermocycler with the thermal profile shown below:

initial denaturation	95 °C	5 minutes
amplification (35 cycles)	92 °C	1 minute
	62 °C	1 minute
	72 °C	30 seconds
final synthesis	72 °C	5 minutes

The species listed in Table 1 were tested for identification of the Enterobacteriaceae family. The primer combinations used and the primer-specific parameters are listed in Table 7. When more than one forward or reverse primer is listed in Table 7, it indicates use of that mixture.

The result of the PCR was analyzed by agarose gel electrophoresis and staining with ethidium bromide. The presence of PCR products indicates the presence of enterobacteria.

The synthesized PCR products are mostly of sizes on the order of 400 to 750 base pairs. Many bands can occur throughout, because ribosomal alleles are heterogeneous in many bacterial species. Table 1 shows the results obtained. They show that the enterobacteria are completely delimited from representatives of other taxa.

Example 2): Detection of a bacterial species, with Pantoea dispersa as an example

Genomic DNA can be isolated from pure cultures of bacteria by standard procedures which are themselves known. Quantities of about 1 to 100 ng each from these preparations can be used in a PCR. The reaction solution can then have the following composition:

genomic DNA	1 µl
H_2O	19.8 µl
Buffer (10x)*1	2.5 µl
dNTP (10 mM)*2	0.25 µl
forward primer A (10 µM)*3	0.20 µl

reverse primer (10 µM)*3	0.20 µl
$MgCl_2$	0.75 µl
Taq polymerase (5 U/µl)*1	0.3 µl

*1: Buffer and enzyme from Biomaster.

*2: Nucleotides from Boehringer Mannheim or any other source.

*3: Equimolar quantities of primers.
In the case of mixtures, each forward and reverse primer has a total final concentration of 10 µM.

The primer combinations SEQ ID 2 + primer x1, SEQ ID (3-6) + primer x1, or the sequence complementary to primer x1 + the sequence complementary to SEQ ID 147 can be used to detect Pantoea dispersa. Here primer x1 is equivalent to the nucleotide CGTTGCCCCGCTCGCGCCGCTCAGTCAC. Primer x1 is a partial sequence from SEQ ID 108.

The PCR can be done in a Perkin Elmer Thermocycler with the thermoprofile shown below:

initial denaturation	95 °C	5 minutes
amplification (35 cycles)	92 °C	1 minute
	62 °C	1 minute
	72 °C	20 seconds
final synthesis	72 °C	5 minutes

The result of the PCR can be made visible by agarose gel electrophoresis and staining with ethidium bromide. The synthesized PCR products have sizes on the order of 370, 320 and 70 base pairs. The absence of amplificates indicates absence of genomic DNA from Pantoea dispersa. This experimental system can give the results summarized in Table 2.

Example 3): Use of a consensus PCR in chip technology

3a) Principle of consensus PCR

In a consensus PCR, such as is shown schematically in Figure 8, at least two "consensus primers" (A1, A2) are used, which can detect DNA from at least two taxonomic units. Those units can be strains, species, or even higher taxonomic units such as kingdoms or classes. In the detection system, the amplified taxonomic units are subsequently differentiated, in at least a second detection step, using another PCR and/or with probes. The PCR primers (B1, B2) of the second, or subsequent, amplification step are each chosen so that they are within the amplification product and have the potential to detect a specific taxonomic unit. By use of more primers (C, D, E . . .), a pool of many taxonomic units can, if necessary, be narrowed down simultaneously. Furthermore, the detection potential can be extended to more taxonomic units in a multiplex mixture (such as A1a, A1b, A1c . . .).The latter case exists if individual nucleotides in a primer differ or if the primers are completely different. The nomenclature of the consensus primers can also be found in the legend for Figure 8.

Amplification products can be identified by means of the primers. The detection is positive if the primers recognize the target DNA and successfully amplify it. In addition probes can provide a specific detection. They hybridize specifically to the amplified DNA and allow a certain DNA sequence to be detected by direct or indirect coupling to dyes. Everything considered, probes can be used in many technical embodiments known to those skilled in the art. For example, there are Southern Blotting, the lightcycler technology with fluorescent probes, or the chip technology, in which arbitrarily many probes are arranged in a microarray.

It is particularly advantageous for success of a consensus PCR that the primers become increasingly specific in the order A, B, C . . . . That can be assured by selection of the DNA target region as shown in Figure 2.

Consensus PCR has the advantage that it allows simultaneous detection of more than two taxonomic units from just a single nucleic acid sample, which can be correspondingly small. The number of detectable microorganisms can be increased in various ways. For instance, the detection potential of a consensus system increases

with the number of primer species A, B, C, or A1a, A1b, A1c, . . . as they are defined in Figure 8. In addition, a PCR solution can, after an initial process with a primer pair A1, A2, be separated and amplified in separate solutions with additional primer pairs B1a + B2a on the one hand and B1b + B2b on the other hand. Finally, the identity of PCR amplificates can be determined by hybridizing with probes.

3b) Example of detection a group of genera of the enterobacteria.

Genomic DNA can be isolated from pure cultures of bacteria by standard procedures which are themselves known. Quantities of about 1 to 100 ng each from these preparations can be used in a PCR. The reaction solution can have the following composition:

genomic DNA	1 µl
H_2O	19.8 µl
Buffer (10x)*1	2.5 µl
dNTP (10 mM)*2	0.25 µl
forward primer A (10 µM)*3	0.20 µl
reverse primer (10 µM)*3	0.20 µl
$MgCl_2$	0.75 µl
Taq polymerase (5 U/µl)*1	0.3 µl

*1: Buffer and enzyme from Biomaster.

*2: Nucleotides from Boehringer Mannheim or any other source.

*3: Equimolar quantities of primers.
In the case of mixtures, each forward and reverse primer has a total final concentration of 10 µM.

As chip technology generally uses very small reaction volumes, the reaction solution shown above can be made smaller with the concentrations remaining constant. It may be necessary to adjust the PCR cycle times. A ribosomal DNA fragment can be amplified initially for consensus PCR. That process can be specific for larger taxonomic units, as described in Example 1, with use of the primers described there. Alternatively, a ribosomal DNA fragment from all bacteria can be amplified. For

instance, use of the primer combination SEQ ID 211 + SEQ ID 212 provides ribosomal DNA of a very broad taxonomic spectrum of bacteria.

The amplified DNA is denatured by standard procedures, thus being converted into single-strand DNA. This form is able to bind to a DNA, RNA, or PNA probe. Then the hybridization of the amplificate is detected with the probe, depending on the design of the chip. Alternatively, detection can be done with an ELISA. The composition of the probe is such that it provides the specificity to meet the requirements. Accordingly, strains, genera, or larger taxonomic units can be detected.

Table 3 shows an example of detection of a group of genera of the family of the enterobacteria using the probe GTTCCGAGATTGGTT as a subsequence of SEQ ID 164. Such a group detection is particularly practical in chip technology if various group detections intersect with each other. Then an individual species, or groups of species, such as those important for food examinations, can be detected in the intersection.

3c) Use of consensus PCR to detect all bacteria

To detect all bacteria, strongly conserved consensus primers are used in a first round of amplification . Suitable for selecting sequences are regions which are peripheral in the ribosomal segment, as shown in Figure 2, are . They are consequently homologous to the regions of SEQ ID 1 beginning at position 2571 or ending at position 3112. From this region, for example, the primers SEQ ID 211 (as primer A1a, for instance) and SEQ ID 212 (as primer A2A, for instance) are particularly suitable for general amplification. Other primers (A1b, A1c, . . ., or A2b, A2c . . .) which cover an arbitrarily large taxonomic range of the Eubacteria in a multiplex PCR can also be derived easily. In this nomenclature, primers A1 and A2 are primer pairs; B and C . . . are nested primers; and A1a and A1b are homologous or similar primers.

An initial differentiation can be accomplished by using nested primers (B, C, D . . .). That can also be supported by dividing the primary PCR solution so that one primer pair B or C or D, etc., is used in each separate PCR solution. This nesting is particularly advantageous because the ribosomal region as shown in Figure 8 increases in variability from the outside to the inside, as is also described in Table 6.

Then it is preferable to use probes for final differentiation and identification. For instance, if species or strains are to be detected, then the probe should hybridize centrally in region 7 as shown in Figure 2.

Table 8 presents many polynucleotides for detection of genera and species or strains in a consensus PCR. Use of primer number 1 from Table 8 has already been described extensively in Example 1.

The properties of the polynucleotides follow their characterization from Table 6 or Figure 2. That means that primer A1 can be assigned to region 1 of Table 6 or Figure 2; primer A2 can be assigned to region 2 ...; primer B2 can be assigned to region 8, and primer A2 to region 9. According to this concept, primers A1-G1 from Table 8 can be used as forward primers, while primers B2 and A2 can be used as reverse primers. For that purpose, the sequences for the two latter primer types must be converted (Exception No. 1, Table 8). The "H1 primers" in particular can be used as genus-specific or species-specific probes.

The plan for a consensus PCR described here is not absolutely necessary for successful detection. In principle, the polynucleotides listed in Table 8 can be used in any arbitrary combination. In practice, one must first decide which bacteria are to be excluded from the detection as "undesired". Then a simpler PCR version that differs from the plan shown can be selected, depending on the objective. The simplest form of consensus PCR, then, consists of just two primers corresponding to the sequences from Table 8, or sequences complementary to them.

Many of the conserved primers listed in Table 8 have the potential to detect the DNA of higher taxonomic units, such as classes, phyla, or families. As can be seen from Table 6, that applies particularly to the peripheral primer A or homologous sequences of SEQ ID 211 + SEQ ID 212. Table 8 shows a broader potential for detecting one or more genera or species, particularly due to the redundant enumeration of the sequences. If only one sequence is explicitly listed for a genus, then two primers from that sequence can be selected for detection. It is also possible to select general primers, such as primer A of related genera, for the bacterial class of concern, and to sketch out a specific sequence, such as "primer h1" for a probe. As long as the sequences are very long, nucleotide fragments at least 15 bases long can be selected from them.

3d) Design of a consensus PCR for chip technology

The actual design of a consensus PCR is determined essentially by the expected number of taxonomic units to be detected. As consensus PCR in its most complex form is also a multiplex PCR, only a limited number of bacteria can be determined in one reaction solution. Experience shows that this number is less than 20. For that reason, it can be advantageous to do different PCR solutions with the same probe and different primers A, B, etc. (nomenclature as shown in Figure 8).

First, bacteria from natural samples are enriched, or genomic DNA is isolated directly from them by standard procedures which are themselves known. Quantities of about 1 to 100 ng each from these preparations can be used in a PCR. The reaction solution can then have the following composition:

genomic DNA	1 µl
H_2O	19.8 µl
Buffer (10x)*1	2.5 µl
dNTP (10 mM)*2	0.25 µl
forward primer A (10 µM)*3	0.20 µl
reverse primer (10 µM)*3	0.20 µl
$MgCl_2$	0.75 µl
Taq polymerase (5 U/µl)*1	0.3 µl

*1: Buffer and enzyme from Biomaster.

*2: Nucleotides from Boehringer Mannheim or any other source.

*3: Equimolar quantities of primers.. In the case of mixtures, each forward and reverse primer has a total final concentration of 10 µM. For example, primers can be designed and combined as described in 3c.

As very small reaction volumes are generally used in chip technology, the reaction solution above can be reduced in volume with the concentrations kept constant. Adjustment of the PCR cycle times may be necessary.

After the amplification rounds, the DNA is combined. Probes, which, in one specific embodiment, can be selected from the column "Primer H1" of Table 8 are immobilized on a chip. Technological procedures for that are known to those skilled in the art. The combined DNA is diluted 1:1 with denaturation buffer (Example 4) and incubated for one hour at room temperature. Then ten times that volume of hybridization buffer (Example 4) is added and the solution is slowly passed over the chip, i. e., the surface with probes adhering to it, at 37 – 60 °C. After this procedure, the chip surface is washed three times for at least 2 minutes with wash buffer (Example 4) at 37 – 60 °C. Then the detection can be done. Primers coupled to a fluorescent dye can be used for that. The fluorescence can be detected with a detector such as a CCD camera. However, there are various alternative possibilities for detection. For instance, it is also possible to follow and quantify the bonding of the single-stranded amplification products to the probes by surface plasmon resonance (SPR) spectroscopy. The latter method has the advantage that no dye need be used for detection. If SPR is used, it should be designed so that detection occurs simultaneously on the regions of the surface which have the same probes. A particularly advantageous embodiment has many (i. e., more than 100 or 1000) separate detection surfaces arranged on the chip. An increase in the SPR signal, caused by the nucleic acid hybridization on these surfaces, is a positive result. The primers listed in Table 8 can be used in this manner to detect the corresponding bacteria; or, in principle, to detect, and if required to quantify, all bacteria.

Example 4) Detection of microorganisms with probes

Probes, being polynucleotides, i. e., DNA, RNA, PNA, or a similar embodiment known to those skilled in the art, are basically suitable for carrying out concentration and detection of DNA or RNA. They occur as single-stranded molecules, or they are converted to the single-stranded form by denaturation, such as by heating or by sodium hydroxide, according to published standard procedures.

To detect microorganisms, the DNA or RNA must be isolated from them and perhaps purified. Various measures can provide high efficiency in the nucleic acid yield:

1) The microorganisms can be concentrated by physical methods, such as with antibodies coupled to magnetic particles, or by centrifuging.

2) The DNA or RNA from the microorganisms can be amplified in a PCR or comparable amplification reaction.

3) The DNA or RNA of the microorganisms, possibly amplified, is concentrated with commercially available material in the course of purification.

Improvement in the efficiency of nucleic acid yields, particularly through amplification, can itself contribute significantly to the specificity of bacterial detection.

This is followed by an incubation step, in which the probes form a hybrid molecule with the nucleic acids to be detected (if the microorganisms to be detected were present). The hybrid molecules are formed under controlled conditions. Then washing steps with buffers follow under conditions (pH, temperature, ionic strength) which allow specific hybridization of nucleic acids while less specific and undesired hybrid molecules dissociate.

Finally the hybrid molecules are detected. There are numerous procedures for detection, which are known in detail to those skilled in the art. Dyes, possibly fluorescent dyes, are used, which are coupled directly or indirectly to the probes or to the DNA being detected, or are incorporated into them. In particular, that can also happen in chip technology or in lightcycler technology. There are also other physical procedures, such as attenuated total reflection of light at interfaces with two different densities, which can be used in detection of hybrid molecules.

Evaluation of the detection can be done in various ways. In an "all or nothing" detection, the hybrid molecule can be detected only if the microorganism being sought were present. That is, if the previously mentioned amplification reaction with the nucleic acids of the microorganisms did not cause any multiplication of the amino acids, then no hybrid molecules will be detectable. However, if "undesired" nucleic acids were amplified, or if they had been present in large quantity, those nucleic acids can be excluded by the stringency conditions in hybridization. Also, quantification of the hybrid molecules allows fine tuning of the specificity of the detection, by establishing a limit for positive detection.

- All the nucleic acids specified in this patent are basically usable as probes. In particular, Table 3 lists an extract of possible probes. The nucleic acids provide detection of the genera specified in the table, and distinction from all other genera of the Eubacteria.

Examples are presented in the following of how the DNA regions specified for this purpose can be used as probes to detect microorganisms. An ELISA detection procedure is used in this example. In that procedure, nucleic acids are detected by an enzymatic reaction which proceeds in microtiter plates.

In this example, the DNA is first amplified in a PCR reaction. That reaction employs primers coupled with digoxigenin. Then a microtiter plate coated with streptavidin is loaded with a biotin-labeled probe, so that the probes couple to the plate surface. The PCR amplificates, denatured by base, hybridize with the probes in a 30-minute reaction. The end of the amplificate that is labeled with 5'dioxigenin now acts as the antigen for a specific antibody which is, in turn, coupled to the enzyme peroxidase. After addition of tetramethylbenzidine, a blue dye forms. Formation of the dye is stopped with 0.5 M sulfuric acid. At the same time, the color turns yellow because of the pH change. The intensity of the absorption is measured at 450 nm in an ELISA reader.

The following reagents are used to perform the ELISA:

- Hybridization buffer (2.5 x SSC)

2.5 x SSC	62.5 ml of 20 x SSC (see below)
2 x Denhardts	20 ml of 50 x Denhardts (see below)
10 mM Tris (Gibco, No. 15504-038)	5 ml of 1 M Tris
1 mM EDTA (Fluka, No. 03699)	1 ml of 0.5 M EDTA

Make up to 0.5 liter with double-distilled water and adjust to pH 7.5.

- Wash buffer 1

1 x SSC	50 ml of 20 x SSC (see below)
2 x Denhardts	40 ml of 50 x Denhardts (see below)
10 mM Tris (Gibco, No. 15504-038)	10 ml of 1 M Tris

1 mM EDTA (Fluka, No. 03699)	2 ml of 0.5 M EDTA

Make up to 1 liter with double-distilled water and adjust to pH 7.5.

- Wash buffer 2

100 mM Tris Gibco, No. 15504-038)	12.15 g
150 mM NaCl (Merck, No. 6404.5000)	8.78 g
0.05% Tween 20 (Serva, No. 37470)	0.5 g
0.5% blocking reagent (Boehringer)	Dissolve 5 g in D1 (see below) at 60 °C.
10 µg/ml herring sperm	10 ml of the 10 mg/ml stock solution

Dilute to 1 liter with double-distilled water and adjust to pH 7.5

- Denaturation buffer

125 mM NaOH (Fluka, No. 71690)	0.5 g
20 mM EDTA (Fluka, No. 03699)	0.745 g

Make up to 0.1 liter with double-distilled water.

- Coupling buffer

10 mM Tris (Gibco, No. 15504-038)	10 ml of 1 M Tris
1 mM EDTA (Fluka, No. 03699)	2 ml of 0.5 M EDTA
100 mM NaCl (Merck, No. 6404.5000)	5.88 g
0.15% Triton X 100 (Chemical storeroom)	15 ml

Make up to 1 liter with double-distilled water and adjust to pH 7.5.

- Stop reagent (0.5 M H_2SO_4)

95% H_2SO_4	14 ml

Make up to 0.5 liter with double-distilled water.

- 50 x Denhardts

Ficoll 400 (Pharmacia Biotech, No. 17-0400-01)	5 g
Polyvinylpyrrolidone (Sigma, No. P-2307)	5 g
Bovine serum albumin	5 g

Make up to 0.5 liter with double-distilled water.

- 20 x SSC

NaCl (Merck, No. 106404.1000)	350.36 g
Sodium citrate (trisodium citrate, dihydrate, Fluka No. 71404)	176.29 g

Make up to 2 liters with double-distilled water and adjust to pH 7.0.

- D 1

100 mM maleic acid (Fluka, No. 63190)	11.62 g
150 mM NaCl (Merck, No. 106404.1000)	8.76 g
NaOH (Fluka, No. 71690)	ca. 7.5 g

Make up to 2 liters with double-distilled water and adjust to pH 7.0.

ELISA procedure:

200 µl binding buffer and 1 µl probe are applied for each well. The microtiter plate is covered with an adhesive film and left to stand for two hours at room temperature. The PCR amplificates to be examined are thawed at room temperature, mixed with the denaturation bufffer in the ratio of 1:1, and incubated for 10 minutes at room temperature. Then 10 ml of this probe is placed into the wells, which have been emptied in the meantime. In addition, 100 µl hybridization buffer is added to each well and incubated for 30 minutes at 37 – 60 °C. To wash, the wells are emptied, filled with 200 ml wash buffer 1 which has been preheated to 37 – 60 °C, and incubated for 2 minutes at the same temperature. This washing step is done three times.

After the wash buffer has been carefully removed, the Anti-Dig-POD-antibody (DAKO) is diluted 1:3000 (1 ml in 3 ml wash buffer 2), and 100 ml of this solution is placed into each of the dry wells. This arrangement is incubated in the incubator at 37 °C for 30 minutes.

Then the microtiter plate is washed three times with 200 ml wash buffer 2 per depression. Then 100 ml of the BM Blue dye (Boehringer) is added per well. After 15 minutes the reaction is stopped by addition of 100 ml 0.5 M H_2SO_4. The absorbance of the samples is measured in the ELISA reader.

The probes listed in Table 4 can be used to detect the species listed in the procedure described above.

Example 5): General usefulness of the DNA regions specified in this patent for detecting bacteria

The ribosomal DNA regions specified here are suitable for detecting eubacteria, especially if they are combined with the 23 S – 5 S ribosomal spacers. One skilled in the art can rapidly identify bacterial taxonomic units of his choice using the sequences under SEQ ID 1-530 or by focusing on the specified ribosomal DNA region. In the following, one possible way is exemplified which shows the general usefulness of this invention for all eubacterial species.

The path described here comprises essentially 3 steps. In the first step, a ribosomal region comprising approximately the last 330 – 430 nucleotides of the 23 S gene, the following transcribed spacer, and the ribosomal 5 S gene is amplified. As this region is of variable length in the various eubacterial species, it has a total length of 400 to about 750 nucleotides. If the DNA sequence is not yet known, it can be advantageous to determine it for the species to be detected and for some closely related species from which it must be distinguished. From a sequence comparison, one skilled in the art can easily determine the best oligonucleotides for the desired detection, e. g., serving as a PCR primer or as a probe. In this example, both primers and probes are selected in that manner. Alternatively, the sequences specified here can also be used directly for a wide spectrum of bacteria, especially if the stringency conditions for the PCR and/or for the hybridization are properly selected.

A) Amplification of ribosomal DNA

The DNA segment to be used can be amplified from genomic bacterial DNA of the proteobacteria and many other bacterial classes with the primers SEQ ID 211 and 212. If other classes present problems in the DNA amplification, use of primers derived from DNA regions corresponding to SEQ ID 211 and 212 will be successful.

Genomic DNA is isolated from pure cultures of the bacteria listed in Table 5 by standard procedures which are themselves known. Quantities of about 1 to 100 ng each from these preparations are used in a PCR. The reaction solution has the following composition:

genomic DNA	1 µl
H_2O	19.8 µl
Buffer (10x)*1	2.5 µl
dNTP (10 mM)*2	0.25 µl
forward primer A (10 µM)*3	0.20 µl
reverse primer (10 µM)*3	0.20 µl
$MgCl_2$	0.75 µl
Taq polymerase (5 U/µl)*1	0.3 µl

*1: Buffer and enzyme from Biomaster or any other source.

*2: Nucleotides from Boehringer Mannheim or any other source.

*3: Equimolar quantities of primers.
In the case of mixtures, each forward and reverse primer has a total final concentration of 10 µM.

The PCR is done in a Perkin Elmer 9600 Thermocycler with the thermoprofile shown below:

initial denaturation	95 °C	5 minutes
amplification (35 cycles)	92 °C	1 minute
	52 °C	1 minute
	72 °C	30 seconds
final synthesis	72 °C	5 minutes

Examples of genomic DNA which can be used for amplification are listed in Table 5.

B) Genus-specific and species-specific amplification of a subregion of the product from A.

The DNA product amplified in A) can be used directly to detect bacteria, especially if specific probes are used. It can be advantageous to amplify primarily a subregion of this sequence if the process is intended to provide limitation to a smaller systematic unit of the bacteria, such as species, genera or families. At least part of the differentiating ability can then be provided already by the amplification primer. The region amplified in A) provides many subregions with specific differentiation capabilities. One skilled in the art can easily recognize those regions by comparing the sequences of bacteria to be identified with closely related bacteria.

In this example, the beginning of the 23 S – 5 S transcribed spacer and the end of it were selected as regions for specific primers. The actual sequences and the origin of the primer are summarized in Table 5. Comparison of the sequences shows that they basically provide a species-specific detection already. The primers for the Vibrio species are exceptions, allowing a genus-specific detection. In the forward primers, the sequence CGAAG...TTTT is conserved, in particular for enterobacteria, and in the reverse primers the sequence AACAGAATTT is conserved. Now there are two possibilities for expanding the specificity of the primers to genera and groups of genera, of the Enterobacteria, for instance. One is to lower the annealing temperatures in the PCR. The other is to shift the sequences for the forward primers toward the 23 S gene, and those for the reverse primers toward the 5 S gene. The result is primers in which the sequences are less variable by species. The actual design, then, can be directed to the requirements for detection. Here, we provide examples of the species-specific detection with the primers of Table 5 by PCR amplification.

Genomic DNA is isolated from pure cultures of the bacteria listed in Table 5 by standard procedures which are themselves known. Quantities of about 1 to 100 ng each from these preparations are used in a PCR. The reaction solution has the following composition:

genomic DNA	1 µl
H_2O	19.8 µl
Buffer (10x)*1	2.5 µl
dNTP (10 mM)*2	0.25 µl
forward primer (10 µM)*3	0.20 µl
reverse primer* (10 µM)*3	0.20 µl
$MgCl_2$	0.75 µl
Taq polymerase (5 U/µl)*1	0.3 µl

*1: Buffer and enzyme from Biomaster or any other source.

*2: Nucleotides from Boehringer Mannheim or any other source.

3: Forward primer A and reverse primers are listed in Table 5. In the case of mixtures, each forward and reverse primer has a total final concentration of 10 µM. Reverse primers* have the sequence complementary to the reverse primers shown in Table 5.

The PCR is done in a Perkin Elmer 9600 Thermocycler with the thermoprofile shown below:

initial denaturation	95 °C	5 minutes
amplification (35 cycles)	92 °C	1 minute
	*45 - 72 °C	1 minute
	72 °C	30 seconds
final synthesis	72 °C	5 minutes

* The annealing temperature can be determined according to the generally used formulas for PCR primers.

Table 5 shows the result of the amplification, i.e. the species-specific detection of bacteria using the primers of Table 5 leads to identification of the bacteria assigned to those primers in this table. On the other hand, use of more general primers, the design of which was described before, can lead to detection of all enterobacterial genera or to detection of all the genera from the γ branch of the proteobacteria.

C) Making the detection more specific by using primers or probes from the 23 S – 5 S ribosomal spacer.

If DNA of higher taxonomic units was amplified in steps A) and/or B), then further differentiation of the detection can be accomplished by selection of probes. A more variable DNA region, such as a central region of the 23 S – 5 S transcribed spacer can be used for species-specific detection. The probes can be integrated into a chip or used in the lightcycler technology or in an ELISA. In the latter case, the ELISA protocol in Example 4 can be used. Then the results of the species-specific detection of bacteria correspond to the selection of the 23 S – 5 S transcribed spacer, because it has mostly a species-specific sequence region. When the primers from Table 5 are used, with use of the corresponding spacer (column SEQ ID from Table 5), then the species listed in that table can be identified.

Explanations of concepts used:

Derivation of DNA sequences

A polynucleotide or oligonucleotide to be used for detection of taxonomic units can be found and developed by deriving it from one or more DNA sequences. In the case of multiple DNA sequences, alignment of the sequences, i. e., a comparison, is advantageous. Derived oligonucleotides may be identical to the original sequence. They may also be a consensus of numerous variables. In that case, the nucleotides of the polymer are selected according to the components most frequently used, or prevalent, at a certain position of the sequences analyzed. It is also possible to select variables in a sequence being developed according to the definition given for "nucleotide" . The DNA or RNA polymers resulting from these variable sequences are, then, a mixture of molecules exhibiting all the nucleotides allowed at the positions of the variables.

Analogous DNA sequences:

Analogous DNA sequences have the same function, or a similar location, as a specified sequence, but cannot be traced back to the same phylogenetic origin. One example is the transcribed spacer between 5 S rDNA and 23 SD rDNA, if it exhibits no similarity with a transcribed spacer at the same location which is being compared

with it. That is possible because it is often so variable in distantly related organisms that it is no longer possible to establish its phylogenetic evolution or homology. The transcribed spacer above, though, is clearly definable as a DNA sequence and in its function as a transcribed spacer, or in its location, because it begins at the end of the coding region of the 23 S rDNA and ends at the beginning of the 5 S rDNA.

Adjacent Genes:

Genes are adjacent if they are not separated by any other gene or if that is the case for two particular genes for most of the species studied. Separation is said to exist only if there is another gene between two other genes.

Enterobacteria

The Enterobacteria are a family of the γ-branch of the proteobacteria. The concept involves all the taxonomic units of the family, especially the genera Alterococcus, Aquamonas, Aranicola, Arsenophonus, Brenneria, Budvicia, Cedecea, Calymmatobacterium, Citrobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Hafnia, Klebsiella, Kluyvera, Koserella, Leclercia, Moellerella, Morganella, Pantoea, Phlomobacter, Photorhabdus, Plesiomonas, Proteus, Providencia, Rahnella, Salmonella, Serratia, Shigella, Wigglesworthia, Xenorhabdus, Yersinia, and Yokenella.

Eubacteria:

The Eubacteria, along with the Archaebacteria, make up a kingdom of the Prokaryotes. Here "bacteria" and "eubacteria" are used synonymously. The concept includes all the taxonomic units within this kingdom. The Eubacteria include, for instance, the Aquificales, Aquificaceae, Desulfurobacterium group, Chlamydiales, Verrumicrobia group, Chlamydiaceae, Simkaniaceae, Waddliaceae, Verrumicrobia, Verrumicrobiales, Coprothermobacter group, Cyanobacteria, Chroococcales, Nostocales, Oscillatoriales, Pleurocapsales, Prochlorophytes, Stigonematales, Cytophagales, the green sulfur bacteria group, Bacteroidaceae, Cytophagaceae, Flavobacteriaceae, Flexibacter group, Hymenobacter group, Rhodothermus group, Saprospira group, Sphingobacteriaceae, Succinovibrionaceae, green sulfur bacteria, Fibrobacter, Acidobacterium group, Fibrobacter group, Firmicutes, Actinobacteria,

Acidomicrobidae, Actinobacteridae, Coriobacteridae, Rubrobacteridae, Sphaerobacteridae, Bacillus group, Clostridium group, Lactobacillus group, Streptococcus group, Clostridiaceae, Haloanaerobiales, Heliobacterium group, Mollicutes, Sporomusa branch, Syntrophomonas group, Thermoanaerobacter group, Flexistipes group, Fusobacteria, green non-sulfur bacteria, Chloroflexaceae group, Chloroflexaceae, photosynthetic Flexibacteria, Holophaga group, Nitrospira group, Planctomycetales, Planctomycetaceae, Proteobacteria, purple non-sulfur bacteria, alpha subdivision of the proteobacteria, beta subdivision of the proteobacteria, gamma subdivision of the proteobacteria, delta/epsilon subdivision of the proteobacteria, Spirochetales, Leptospiraceae, Spirochaetaceae, Synergistes group, Thermodesulfobacterium grup, Thermotogales, Thermus group or the Deinococcus group.

Gene:

The gene comprises the open reading frame or coding region of a DNA. Thus it codes solely for a single protein. The cistron is also a gene, but it, along with other cistrons, is on a mRNA. DNA regions which regulate transcription of the gene, such as promotors, terminators, and enhancers, are also part of the gene. When, in this patent, we speak, in a simplifying manner of the 23 S rDNA gene and the 5 S rDNA gene, this is based on the usual designations. According to our definition, though, the 23 S rDNA gene or the 5 S rDNA gene is not a gene but an independent functional DNA segment, because it does not code for a protein and cannot be subdivided into codons.

Transcribed spacer:

The transcribed spacer, on which we focus here, lies behind the coding region of the 23 S rDNA gene and before the coding region of the 5 S rDNA gene. In its systematic classification, it has a special position. Because it is transcribed, and thus is part of the mRNA and a biologically inactive precursor molecule, preRNA, it is not part of the intergene region. The precursor molecule is converted into a biologically active molecule in the ribosomal context by excising the transcribed spacer. On the other hand, it cannot be assigned functionally or phylogenetically to the 23 S gene or the 5 S gene. As the gene concept apparently cannot be utilized for classification in this

case, let the "transcribed spacer" of the ribosomal operon be considered an independent functional DNA (RNA) class equivalent to the "gene" and the "intergenic region".

Homologous DNA sequences

DNA or RNA sequences are homologous if they have the same phylogenetic origin. That may be recognizable by the fact that at least 40% of the nucleotides in a DNA segment are identical. There may be variable pieces in a large DNA segment. In that case it is sufficient for the phylogenetic relation to be shown by presence of a sequence 25 nucleotides long, which is at least 60% identical with another sequence, 25 nucleotides long, of the DNA being compared. Also, homologous sequences can frequently best be recognized by comparison with closely related organisms. To recognize homology of sequences of more distantly related organisms, it is then necessary to do a step-by-step comparison with sequences of species which bridge the separation to the distantly related phylogenetic species.

Identical DNA sequences / Percent identity

Subsequences of a larger polynucleotide are considered to determine the identity (in the sense of complete agreement, equivalent to 100% identity) of DNA or RNA sequences. These subsequences comprise 10 nucleotides, and are identical if all 10 components are identical in two comparison sequences. The nucleotides thymidine and uridine are considered identical. All the possible fragments of a larger polynucleotide can be considered as subsequences.

The identity is 90% if 9 of 10 nucleotides, or 18 or 20 nucleotides, are the same in a section on the two sequences being compared.

As an example, consider two polynucleotides made up of 20 nucleotides, which differ at the 5th component. In a sequence comparison, then one would find six 10-element nucleotides which are identical and 5 which are not identical because they differ in one component.

The identity can also be determined by degrees, with the unit reported being a percentage. To determine the degree of identity such subsequences are considered

that comprise at least the length of the sequence actually used, e.g. as a primer, or 20 nucleotides.

As an example, we compare polynucleotide A with a length of 100 nucleotides and polynucleotide B with a length of 200 nucleotides. A primer is derived from polynucleotide B with a length of 14 nucleotides. To determine the degree of identity, polynucleotide A is compared with the primer over its entire length. If the sequence of the primer occurs in polynucleotide A, but with a difference in one component, then we have a fragment with a degree of identity of 13/14, or 92.3%.

As a second example, the two polynucleotides above, A and B, are compared in their entirety. In this case, all the possible comparison windows with lengths of 20 nucleotides are applied and their degrees of identity are determined. Then if nucleotides numbered 50 – 69 of polynucleotides A and B are identical except for nucleotide number 55, then these fragments have a degree of identity of 19/20 or 95%.

Conserved and variable primers

Conserved primers are nucleotides which hybridize with conserved DNA or RNA regions. The concept 'conserved' characterizes the evolutionary variability of a nucleotide sequence for species of various taxonomic units. Therefore it is a measure of comparison. Depending on which sequence is used for comparison, a region or primer can be conserved or variable. Characterization of a primer as "conserved" or "variable" is accomplished by means of directly adjacent or overlapping regions with respect to the of hybridization target, which have the same length as the primer. Therefore one can select comparison sequences from the same organism, or homologous or similar segments from different organisms. When two sequences are compared, one is conserved if it is at least 95% identical with the comparison sequence, or variable if it is less than 95% identical.

Nested primers

Nested primers are used particularly in consensus PCR. These are primers which amplify a fragment of an already amplified polynucleotide. Therefore nested primers hybridize with a region within an already multiplied DNA or RNA target molecule.

Amplification with nested primers can be done as frequently as desired, giving successively smaller amplification products.

Hybridization of DNA or RNA

Two identical or similar nucleotide fragments can hybridize with each other to form a double strand. Such hybridization does not occur only between DNA, RNA, or PNA single strands. It is also possible for hybrid molecules to form between DNA and RNA, DNA and PNA, RNA and PNA, etc. There are numerous factors which determine whether two polynucleotides hybridize. Hybridization can take place in a temperature range of, preferably, 37 – 60 °C. Hybridization can also occur in discrete hybridization and washing steps. Example 4) presents experimental parameters to make hybridization conditions more specific. Specific hybridization takes place if only a single hybridization with the desired target sequence occurs with the probe used and not with any other DNA which is also in the sample.

Combinations in use of nucleotides

Primers, probes, DNA fragments, subregions of polynucleotides or oligonucleotides can be used in many combinations. Possibilities include, for instance, arbitrary combination of two primers from a group of primers; arbitrary selection of one probe from a group of sequences; and selection of primers from the same group of sequences. In the latter cases the primer and probe(s) may be identical or different. Primers or probes can also be made up of two or more DNA fragments, with all possible variations in the composition being eligible. Combinations are also possible in the sequence of distinct PCR steps with different primers and the use of probes.

Consensus PCR

A consensus PCR is carried out with consensus primers. These are able to amplify the DNA of at least 2 taxonomic units (of all taxonomic units in the ideal case). In subsequent analysis steps, the identity of the amplified DNA is determined. For this purpose, either other PCR steps are done, which discriminate between smaller taxonomic units with variable nested primers if necessary, or the final determination of a taxonomic unit can be done with specific probes rather than with variable primers.

Nucleotides

Nucleotides are the building blocks of DNA or RNA. The abbreviations mean: G = guanosine, A = adenosine, T = thymidine, C = cytidine, R = G or A; Y = C or T; K = G or T; W = A or T; S = C or G; M = A or C; B = C, G or T; D = A, G or T; H = A, C or T; V = A, C, or G; N = A, C, G. or T; I = inosine.

Taxonomic units

Taxonomic units of bacteria are all the known taxonomic subdivisions, such as kingdoms, classes, phyla, orders, families, genera, species, strains, intermediates of those taxonomic units such as subclasses, suborders, subfamilies, etc.; or groups of these taxonomic units.

Detailed description of the invention

This invention comprises essentially 5 partial aspects which reflect the invention in its general form and in its special aspects:

- strategic selection of DNA target regions using adjacent genes
- description of use of a ribosomal DNA region from the end of the 23 S rDNA, the transcribed spacer, and parts of the 5 S rDNA to detect all bacteria
- provision of primers and probes for many bacteria
- detection of the families of the enterobacteria and their members
- use of a consensus PCR to detect all bacteria

Strategic selection of DNA target regions using adjacent genes

The invention consists in the use of portions of adjacent genes to detect taxonomic units, i. e., kingdoms, classes, phyla, families, genera and strains, as well as intermediate forms of these units. The advantage of the invention is that DNA regions which span two genes are very heterogeneous with respect to variability. That has been found, for instance, with the ribosomal operons, especially the 23 S / 5 S rDNA segment. Because of the presence of very strongly conserved regions and very

poorly conserved regions, one skilled in the art is enabled to detect all possible closely and even distantly related organisms.

Description of use of a ribosomal DNA region from the end of the 23 S rDNA, from the transcribed spacer, and from parts of the 5 S rDNA to detect all bacteria

In particular, a 23 S – 5 S rDNA region comprising about 400 – 750 nucleotides can be used to detect bacteria. The latter region consists of about 330 – 430 nucleotides of the terminal region of the 23 S rDNA, the adjoining transcribed spacer, and the 5 S rDNA gene. In individual cases, a t-RNA gene can also be inserted into the spacer and used for the detection. The region described corresponds to the nucleotides 2571 – 3112 of the SEQ ID 1, which represents the 23 S and 5 S rDNA genes of Escherichia coli. The homologous regions, and those corresponding to the above region, from other bacteria can be determined by a sequence comparison known to those skilled in the art. The beginning of the above-described region at the terminus of the 23 S rDNA gene and the end of the 5 S rDNA genes can be determined easily by comparing the ribosomal DNA sequences of two species A and B, especially for members of the same families, or even orders or phyla. Should this not be as easy for a comparison of species A and a more distantly related species C, one arrives at the desired result by making a comparison between the sequences of species B and C, in which B and C should be closely related to each other. In this way, by a series of separate sequence comparisons, it is possible to determine the homogeneous ribosomal regions of the 23 S rDNA, the transcribed spacer, and the 5 S rDNA of all Eubacteria. Because of the variability of individual subregions, length differences of several hundred nucleotides can occur. In addition, this invention allows use of subregions of the region described above. Table 6 describes a large portion of these regions.

Provision of/Providing primers and probes for many bacteria

Along with the general description of the useful rDNA region, sequences (SEQ ID 1-530) are also provided, which can be used to detect bacteria. Depending on the particular objective, the polynucleotides occurring in SEQ ID 1-530 can be used completely, or fragments of the sequence can be used. The sequences specified in

SEQ ID 1-530 are derived from the previously described region of the 23 S rDNA gene, transcribed spacer, and 5 S rDNA gene.

In the technical execution, organisms can be detected by means of the DNA regions and sequences specified for that purpose, using probes and/or primers. Primers are nucleotides which act as starter molecules for the amplification. They deposit on the target sequence, so that the region is synthesized anew using a polymerase. Their specificity can be adjusted by the degree of identity of the primer with the target sequence. The taxonomic specificity is also determined by the selection of the target sequence within the ribosomal region described here (see also Table 6). Primers can thus be used in different ways: For instance, it is possible to amplify the entire region corresponding to Figure 2, or homologous to the nucleotides number 2571-3112 of the SEQ ID 1 (E. coli) with primers SEQ ID 211 and 212. A mixture of more than two primers can also be used to optimize the amplification. Moreover it is possible to select the primer so that only the DNA of certain bacteria is amplified. In this case, then, there are two kinds of information in the case of positive amplification: First, they show the presence of the bacteria sought; and second, they show the identity of the bacteria. By means of sequential amplification steps with nested primers, the information obtained at the end of the DNA synthesis can be adjusted according to the requirements.

In a distinct step, the DNA, which ideally has previously been amplified, is bound to probes, concentrated, and detected. Probes are oligonucleotides or polynucleotides which can bind to single-stranded DNA segments. The affinity of the probes to the target sequence is determined by their degree of identity with it. The hybridization conditions also have a significant effect. That is, the buffer salt concentration, the incubation time, and the incubation temperature must be optimized. One skilled in the art can rapidly optimize those parameters using current methods. Exemplary hybridization conditions are given in the examples. Probes, just like primers, can work in two ways. First, they can show the presence of bacterial DNA or amplification products. Second, they can contribute to the detection of the DNA of specific bacteria. In this duality of their function they resemble the primers. Accordingly, the task of identification of organisms can be divided between primers and probes. Also, the probes, like the primers, derive from freely selectable regions of the terminal region of the 23 S rDNA, of the transcribed spacer, of the 5 S rDNA, or from the entire region.

One special advantage of this invention is that the ribosomal region selected according to Figure 2 is be composed heterogeneously of very variable and very conserved regions, over an extremely broad range. As there are very many combinations in utilization of subregions, e. g., as shown in Table 6, this invention offers the potential of detecting all bacterial species and taxonomic units.

Detection of the familiy of the enterobacteria and their members

Bacterial families such as the Enterobacteriaceae can be detected by using the DNA target regions characterized in this document (Example 1). The enterobacteria are a homogeneous taxonomic unit of the γ branch of the proteobacteria or purple bacteria. They are of particular interest because they include many pathogenic bacteria, such as Escherichia coli (EHEC, etc.), Shigella, Salmonella, and Yersinia. Thus they are suitable marker organisms for examining the hygienic status of foods. In clinical microbiology, detection of enterobacteria can be an initial step in narrowing down or identifying pathogenic microorganisms. From the list contained in this work, for instance, the primer SEQ ID 2-25, in various combinations, is usable for identifying the enterobacteria as the family. Many of the sequences listed are also suitable for identifying individual members of the enterobacteria, i. e., genera, species and strains. Other sequences are also produced for the other taxonomic units of the proteobacteria, especially the entire γ branch, as well as for the Firmicutes. Description of the ribosomal region as shown in Figure 2 shows another way in which one skilled in the art can easily obtain more sequences so as to detect all the Eubacteria.

Use of a consensus PCR to detect all bacteria

One special advantage of our invention is that the DNA target region, as described in Figure 2, can be detected in an ideal manner in a consensus PCR. One significant prerequisite for the experimental applicability of this method is that the sequences become increasingly variable within a target region to be amplified. The region of the ribosomal operon which we have characterized has such a configuration for all the species investigated.

The plan for the consensus PCR is outlined in Figure 8. As a general rule, a "master fragment" is amplified first. That can be the same as the complete fragment as shown in Figure 2, or a part of it. Now if there are various microorganisms to be identified in a sample, this fragment is amplified for all of them. Finally, the individual organisms are identified with specific probes and/or in combination with more PCR steps. The detection with probes can even be miniaturized and accomplished on chips. Alternatively, detection can be done in the classical ELISA procedure. The components for bacterial detection can be prepared in the form of a kit.

Fluorescent dyes are particularly advantageous for detection. They can be coupled to the primers or to the probes. However, non-fluorescent dyes are also used often, particularly in the ELISA or the Southern Blot procedures. Genetrack and Light Cycler technology provides another possibility for detection. In principle, all these methods offer the option of quantitative determination. Thus by evaluating the detection signal it is also possible to ultimately draw conclusions about the number of bacteria in a sample.

Detection of bacteria with this invention can be done in an experimental context that is well known to one skilled in the art. For instance, bacteria can first be enriched in a suitable medium before detection. In working with foods, physical separation steps such as centrifugation or sedimentation are advantageous. It is also possible to enrich the bacteria in such a way that it is later possible to draw conclusions about their initial number. Furthermore, one can do threshold value tests with respect to the bacterial count. All in all, then, quantitative or semiquantitative determination of microorganisms is possible.

The (enriched) bacteria are broken up to isolate the genomic DNA. The procedures for cell disintegration that are well known to one skilled in the art are often based on physical (glass beads, heat) and chemical (NaOH) influences. It is also possible, though, to use cells directly in a PCR to detect DNA. Moreover it can also be advantageous to purify the genomic DNA, especially if it is distributed through a food matrix. These procedures are also known to those skilled in the art. DNA purification kits are also commercially available.

Table 1: Detection of enterobacteria excluding other bacteria (Example 1)

No.	Species	Strain	Detection
1	Budvicia aquatilis	DSM 5025	+
2	Buttiauxella agrestis	DSM 4586	+
3	Cedecea davisae	DSM 4568	+
4	Citrobacter koser	DSM 4595	+
5	Erwinia carotovora	DSM 30168	+
6	Erwinia chrysanthemi	DSM 4610	+
7	Ewingella americana	DSM 4580	+
8	Enterobacter agglomerans	B-5081-1	+
9	Enterobacter aerogenes	DSM 30053	+
10	Enterobacter sakazakii	DSM 4485	+
11	Enterobacter intermedius	DSM 4581	+
12	Enterobacter cloacae	DSM 30054	+
13	E. coli	BC 7883	+
14	E. coli	H123	+
15	E. coli	BC 7884	+
16	E. coli	BC 7885	+
17	E. hermanii	B-4943a	+
18	E. coli	ATCC 8739	+
19	Hafnia alvei	DSM 30163	+
20	Klebsiella pneumoniae	ATCC 13883	+
21	Klebsiella pneumoniae	DSM 2026	+
22	Klebsiella planticola	DSM 4617	+
23	Klebsiella oxytoca	DSM 5175	+
24	Kluyvera cryocrescens	DSM 4583	+
25	Morganella morganii	DSM 30164	+
26	Plesiomonas shigelloides	DSM 8224	+
27	Pantoea ssp.	B-5200	+
28	Pantoea dispersa	DSM 30073	+
29	Proteus rettgeri	DSM 1131	+
30	Proteus rettgeri	ATCC 14505	+
31	Providencia stuartii	DSM 4539	+
32	Rahnella aquatilis	DSM 4594	+
33	Rahnella aquatilis	DSM 4594	+
34	Serratia protcamaculans	DSM 4487	+
35	Serratia ficaria	DSM 4509	+

Table 1: Detection of enterobacteria excluding other bacteria (Example 1)
- Continuation -

No.	Species	Strain	Detection
36	Serratia plymutica	DSM 49	+
37	Serratia rubidea	DSM 4480	+
38	Serratia marcescens	DSM 1636	+
39	Salmonella bongori	DSM 7952	+
40	Yersinia pseudotuberculosis	DSM 8992	+
41	Yersinia pseudotuberculosis	DSM 8992	+
42	Yersinia enterolytica	DSM 4790	+
43	Acinctobacter calcoaceticus	DSM 590	-
44	Aeromonas hydrophila	DSM 6173	-
45	Aeromonas enteropelogenes	DSM 6394	-
46	Fransilla tularensis Isolat	F16	-
47	Franzisella philomiragia	DSM 7535	-
48	Moraxella catarrhalis	DSM 9143	-
49	Pasteurella pneumotropica	B-2397 A 13	-
50	Pseudomonas beyjerinkii	DSM 7218	-
51	Vibrio fischeri	DSM 507	-
52	Vibrio alginolyticus	DSM 2171	-
53	Vibrio proteolyticus	DSM 30189	-
54	Vibrio paramaemolyticus	DSM 10027	-
55	Vibrio harveyi	DSM 6104	-
56	Xanthomonas maltophila	BC 4273	-
57	Achromobacter xylosa	DSM 2402	-
58	Alcaligenes spp	DSM 2625	-
59	Alcaligenes latus	DSM 1122	-
60	Brucella neotomae	ATCC 25840	-
61	Brucella ovis	ATCC 23459	-
62	Enterococcus casseliflavus	DSM 20680	-
63	Flavobacterium sp	ATCC 27551	-
64	Flavobacterium resinovorum	DSM 7438	-
65	Flavobacterium johnsonii	DSM 2064	-
66	Flavobacterium flavense	DSM 1076	-
67	Lactobacillus bifermentans	BC 8463	-
68	Pseudomonas paucimobilis	DSM 1098	-
69	Pseudomonas cepacia	DSM 3134	-
70	Sphingobacterium multivorans	DSM 6175	-

Table 2: Detection of Pantoea dispersa excluding other bacteria (Example 2)

No.	Species	Detection
1	Pantoea dispersa	+
2	Budvicia aquatica	-
3	Buttiauxella agrestis	-
4	Enterobacter agglomerans	-
5	Erwinia carotovora	-
6	Erwinia crysanthemi	-
7	Escherichia coli	-
8	Escherichia vulneris	-
9	Escherichia hermannii	-
10	Hafnia alvei	-
11	Klebsiella oxytoca	-
12	Kluyvera cryoescens	-
13	Morganella morganii	-
14	Proteus mirabilis	-
15	Proteus rettgeri	-
16	Proteus stuartii	-
17	Providencia stuartii	-
18	Rahnella aquatilis	-
19	Serratia ficaria	-
20	Serratia fonticola	-
21	Serratia marcescens	-
22	Serratia plymuthica	-
23	Serratia proteamaculans	-
24	Serratia rubidea	-
25	Yersinia enterolytica	-
26	Yersinia peudotuberculosis	-
27	Acinetobacter calcoaceticus	-
28	Aeromonas enteropelogenes	-
29	Aeromonas hydrophila	-
30	Cedecea davisae	-
31	Haemophilus influenzae	-
32	Moraxella catarrhalis	-

Table 2: Detection of Pantoea dispersa excluding other bacteria (Example 2)
– Continuation -

Nr.	Art	Nachweis
33	Pasteurella pneumotropica	-
34	Stenotrophomonas multophila	-
35	Vibrio alginolyticus	-
36	Vibrio fisheri	-
37	Vibrio harveyi	-
38	Vibrio parahaemolyticus	-
39	Alcaligenes sp.	-
40	Bacillus subtilis	-
41	Brucella abortus	-
42	Brucella ovis	-
43	Flavobacterium resinovorum	-
44	Pseudomonas paucimobilis	-
45	Pseudomonas cepacia	-
46	Ralstonia pickettii	-
47	Sphingobacterium multivorum	-
48	Sphingomonas paucimobilis	-
49	Streptococcus faecalis	-

Table 3: Detection of a group of genera with the probe GTTCCGAGATTGGTT

No.	Species	Detection
1	Rahnella aquatilis	+
2	Serratia ficaria	+
3	Serratia fonticola	+
4	Serratia marcescens	+
5	Serratia plymuthica	+
6	Serratia proteamaculans	+
7	Serratia rubidea	+
8	Yersinia enterolytica	+
9	Yersinia pseudotuberculosis	+
10	Budvicia aquatica	-
11	Buttiauxella agrestis	-
12	Enterobacter agglomerans	-
13	Erwinia carotovora	-
14	Erwinia crysanthemi	-
15	Escherichia coli	-
16	Escherichia vulneris	-
17	Escherichia hermannii	-
18	Hafnia alvei	-
19	Klebsiella oxytoca	-
20	Kluyvera cryoescens	-
21	Morganella morganii	-
22	Pantoea dispersa	-
23	Proteus mirabilis	-
24	Proteus rettgeri	-
25	Proteus stuartii	-
26	Providencia stuartii	-
27	Acinetobacter calcoaceticus	-
28	Aeromonas enteropelogenes	-
29	Aeromonas hydrophila	-

Table 3: Detection of a group of genera with the probe GTTCCGAGATTGGTT

- Continuation -

No.	Species	Detection
30	Cedecea davisae	-
31	Haemophilus influenzae	-
32	Moraxella catarrhalis	-
33	Pasteurella pneumotropica	-
34	Stenotrophomonas multophila	-
35	Vibrio alginolyticus	-
36	Vibrio fisheri	-
37	Vibrio harveyi	-
38	Vibrio parahaemolyticus	-
39	Alcaligenes sp.	-
40	Bacillus subtilis	-
41	Brucella abortus	-
42	Brucella ovis	-
43	Flavobacterium resinovorum	-
44	Pseudomonas paucimobilis	-
45	Pseudomonas cepacia	-
46	Ralstonia pickettii	-
47	Sphingobacterium multivorum	-
48	Sphingomonas paucimobilis	-
49	Streptococcus faecalis	-

Table 4: Specific probes for the detection of bacterial genera and species

No.	Probe SEQ ID	Detection of Genus/Species
1	96	Budvicia aquatica
2	97	Buttiauxella agrestis
3	98	Enterobacter agglomerans
4	99	Erwinia carotovora
5	100	Erwinia chrysanthemi
6	101	Escherichia coli
7	102	Escherichia hermannii
8	103	Escherichia vulneris
9	104	Hafnia alvei
10	105	Klebsiella oxytoca
11	106	Kluyvera cryoescens
12	107	Morganella morganii
13	108, 109	Pantoea
14	110	Proteus mirabilis
15	111	Proteus rettgeri
16	112	Providencia stuartii
17	113	Rahnella aquatilis
18	114	Serratia ficaria
19	115	Serratia fonticola
20	116	Serratia marcescens
21	117	Serratia plymuthica
22	118	Serratia proteamaculans
23	119	Serratia rubidea
24	120	Yersinia enterolytica
25	121	Yersinia pseudotuberculosis
26	122	Acinetobacter calcoaceticus
27	123	Aeromonas enteropelogenes
28	124	Aeromonas hydrophila
29	125	Cedecea davisae
30	126	Haemophilus influenzae
31	127	Moraxella catharralis
32	128	Pasteurella pneumotropica
33	129	Stenotrophomonas multophila

Table 4: Specific probes for the detection of bacterial genera and species

- Continuation 1 / 2 -

No.	Probe SEQ ID	Detection of Genus/Species
34	130	Vibrio alginolyticus
35	131	Vibrio fisheri
36	132	Vibrio harveyi
37	133	Vibrio parahaemolyticus
38	134	Vibrio proteolyticus
39	432	Salmonella typhi
40	433	Buchnera aphidocola
41	434	Pseudomonas stutzeri
42	435	Thiobacillus ferrooxidans
43	436	Agrobacterium vitis
44	437	Adalia bipunctata
45	438	Amycocalatopsis orientalis
46	439	Brucella
47	440	Bradyrhyzobium japonicum
48	441	Pseudomonas paucimobilis
49	442	Rhodobacter sphaeroides
50	443	Rickettsia prowazekii
51	444	Pseudomonas cepacia
52	445	Ralstonia pickettii
53	446	Campylobacter jejuni
54	447	Helicobacter pylori
55	448	Actinoplanes utahensis
56	449	Bacillus halodurans
57	450	Bacillus subtilis
58	451	Clostridium tyrobutyricum
59	452	Frankia
60	453	Microbispora bispora
61	454	Mycobacterium leprae
62	455	Mycobacterium smegmatis
63	456	Mycobacterium tuberculosis
64	457	Mycoplasma gallisepticum

Table 4: Specific probes for the detection of bacterial genera and species

- Continuation 2 / 2 -

No.	Probe SEQ ID	Detection of Genus/Species
65	458	Propionibacterium freudenreichii
66	459	Rhodococcus erythropolis
67	460	Rhodococcus fascians
68	461	Staphylococcus aureus
69	462	Streptococcus faecalis
70	463	Streptomyces ambifaciens
71	464	Streptomyces galbus
72	465	Streptomyces griseus
73	466	Streptomyces lividans
74	467	Streptomyces mashuensis
75	468	Flavobacterium resinovorum
76	469	Sphingobacterium multivorans
77	470	Synechococcus
78	471	Synechocystis
79	472	Borrelia burgdorferi
80	473	Chlamydia trachomatis
81	474	Azotobacter vinelandii
82	475	Cowdria ruminantium
83	476	Mycobacterium intracellulare
84	477	Mycobacterium lufu
85	478	Mycobacterium simiae
86	479	Mycobacterium smegmatis
87	480	Saccharomonospora azurea
88	481	Saccharomonospora caesia
89	482	Saccharomonospora cyanea
90	483	Saccharomonospora glauca
91	484	Saccharomonospora viridis
92	485	Wolbachia pipientis
93	525	Sphingomonas paucimobilis
94	526	Zymomonas mobilis
95	527	Alcaligenes
96	528	Borrelia burgdorferi
97	529	Xanthomonas campestris
98	530	Cowduria ruminantium

Table 5. Primers for detection of bacterial species or genera

No.	Species used	SEQ ID	Forward primer	Reverse primer (reverse primer* = complementary)
1	Budvicia aquatica	96	CGAGGTGTTTTAAGGAAAGTT	CGGTCAATAGACAGAATAT
2	Buttiauxellis agrestis	97	CGAAGGTGTTTTGGTTGAGAG	GGTTGATGAAACAGAATAT
4	Enterobacter agglomerans	98	CGAAGATGTTTTGGCGGATTG	GTTTCTGGCAACAGAATTT
5	Erwinia carotovora	99	CGAAGGTGTTTTGAGAGTGAC	TTGGGATGAAACAGAATTT
6	Erwinia chrysanthemi	100	CGAAGGTGTTTTAGAGAGATT	TCGGGATGAAACAAAATTT
7	Escherichia coli	101	CGAAGCTGTTTTGGCGGATGA	GTCTGATAAAACAGAATTT
8	Escherichia hermannii	102	CAGAGTGGTTTTGGTGTTGCG	CAGCAGGTGAACAGAATTT
9	Escherichia vulneris	103	CGAAGATGTTTTGGCGGATTT	CGTCAGACAGACAGAATTT
10	Hafnia alvei	104	CGAAGGTGTTTTAAGACGCAG	GGTACAAATAACAGAATAT
11	Klebsiella oxytoca	105	CGAAGATGTTTTGGCGATTTG	GTTTCTGACAACAGAATTT
12	Kluyvera cryoescens	106	CAAAGATGTTTTGGTGAAAAG	CGGGTTAATAACAGAATTT
13	Morganella morganii	107	CGAAGGTGTTTTGAGTTGAGA	TTTGGATTGAAATGAATTT
14	Pantoea dispersa	108	CAGAGGCGTTTTGGTCTGAGA	GCGGTNTAAAACAAAATTT
15	Pantoea ssp.	109	CGAAGATGTTTTGGCGGAATG	GTTTCTGGCAACAGAATTT
16	Proteus mirabilis	110	CGAAAGTGTTTTGTCAGAGAG	AGTGATTAAAACCGAATTT
17	Proteus rettgeri	111	CGAAGGTGTTTTAGAGAGATA	CGGGAACAAAACAGAATTT
18	Providencia stuartii	112	CGAAGGTGTTTTAGAGAGACG	ACGGGAACGAACCGAATTT
19	Rahnella aquatilis	113	CGAAGGTGTTTTTGATTTGAG	TATGAATGAAACAGAATTT
20	Salmonella typhi	432	CGAAGGTGTTTTGGAGGATAA	GATAAAAGAAACAGAATTT

Table 5. Primers for detection of bacterial species or genera

- Continued -

No.	Species used	SEQ ID	Forward primer	Reverse primer (reverse primer* = complementary)
21	Serratia ficaria	114	CGAAGGTGTTTTAGAGAGACG	CAAGAATGAAACAGAATTT
22	Serratia fonticola	115	CCAAGGTGTTTTGAAGAGATT	TTGAAATGAAACAGAATTT
23	Serratia marcescens	116	CGAAGGTGTTTTTAGAGAGAT	TTGGAATGAAACAGAATTT
24	Serratia plymuthica	117	CGAAGGTGTTTTAGAGAGATT	TTGGAATGAAACAGAATTT
25	Serratia proteamaculans	118	CAAAGGTGTTTTAGAGAGATT	TTGGAATGAAACANAATTT
26	Serratia rubidea	119	CGAAGGTGTTTTAGAGAGATT	TCGGGATGAAACAGAATTT
27	Yersinia enterolytica	120	CAAAGGTGTTTTGTATTTGAG	GTTAGTTTAGACAGAATTT
28	Acinetobacter calcoaceticus	122	CCAAGCAGTTGTATATAAAGC	GCAACCAATAAGACCAATG
29	Aeromonas enteropelogenes	123	CCAAGAAGTGTTTNTGGTGCT	TTCCAAGATTGAAGATTTT
30	Aeromonas hydrophila	124	CCAAGAAGTGTTCTAAGGCTT	TTCTCAGATTGAAGAATTT
31	Buchnera aphidocola	433	CCAGAGGTGTTTTTTATAAAA	ATCTTGTTTTACTGAATTT
32	Haemophilus influenzae	126	GCTCAAGTGTTTTTGGGAGCT	CGGTCAGTAAACAGAATTT
33	Moraxella catarrhalis	127	ACCCAAGTGGTTTACCACTGA	GTAATAAACAGACTCATAC
34	Pasteurella pneumotropica	128	ACCAAATTTGTTTATCGTAAC	AGTTGTTATAATAAAACAT
35	Vibrio alginolyticus	130	CCAAGGGGTTTTGATGGACTC	TTTCCAGATTAAAGAATTT
36	Vibrio fisheri	131	CCAAGTGGTTTGTATCAAGCA	TTAAGTAAAACAAACACAG
37	Vibrio harveyi	132	CCAAGGGGTTTTGATGGACTC	TTTCCAAATTAAAGAATTT
38	Vibrio parahaemolyticus	133	CCAAGGGGTTTTGATGGACTC	TTTCCGAATTAAAGAATTT
39	Vibrio proteolyticus	134	CCAAGGGGTTTTGATGGACTC	TTGTTCCAGACAAAATTTT

Table 6: Detection potential and specification of the location of DNA fragments from the rDNA operon

No. in Fig. 2	DNA region	Position in SEQ ID 1	Detection potential
1	Terminal region of the 23 S rDNA gene	2667 – 2720	Phyla, classes, orders, families
2	Terminal region of the 23 S rDNA gene	2727 – 2776	Phyla, classes, orders, families
3.	Terminal region of the 23 S rDNA gene	2777 – 2800	Phylas, classes, orders, families
4.	Terminal region of the 23 S rDNA gene	2801 – 2838	Classes, orders, families
5.	End of the 23 S rDNA gene	2857 – 2896	Phyla, classes, orders, families
6.	Beginning of the 23 S – 5 S transcribed spacer	2897 – 2938	Orders, families, genera, species, strains
7.	23 S – 5 S transcribed spacer	2939 – 2983	Genera, species, strains
8.	End of the 23 S – 5 S transcribed spacer	2984 – 2999	Families, genera, species, strains
9.	Beginning of the 5 S rDNA gene	3000 – 3032	Phyla, classes, orders, families

Table 7: Primers from Example 1

Forward primer	Reverse primer	Annealing temperature (°C)	Figure
SEQ ID 2	SEQ ID 7 – 22	62	3
SEQ ID 2	SEQ ID 23 – 24	62	4
SEQ ID 2	SEQ ID 25	67	5
SEQ ID 3 – 6	SEQ ID 23 – 24	62	6
SEQ ID 3 – 6	SEQ ID 25	67	7

Table 8. Consensus PCR for detection of bacteria

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer H1 SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
1	Enterobakterien	1	7-22							4	5
2	Enterobakterien	26	34	42	54	66	78	85			135
3	Acinetobacter	27	35	43	55	67	79				
4	Aeromonas	28	36	44	56	68	80	87			155
5	Haemophilus	29	37	45	57	69	81				
6	Moraxella	30	38	46	58	70	82				
7	Pasteurella	31	39	47	59						
8	Stenotrophomonas	32	40	48	60	72		90			
9	Vibrio	33	41								
10	Vibrio alginolyticus			49	61	73		91	130		160
11	Vibrio fisheri			50	62	74		92	131		161
12	Vibrio harveyi			51	63	75		93	132		162
13	Vibrio parahaemolyticus			52	64	76		94	133		163
14	Vibrio proteolyticus			53	65	77		95	134		163
15	Pasteurella pneumotropica					71	83		128		158
16	Acinetobacter calcoaceticus							86	122		154
17	Haemophilus influenzae							88	126		156
18	Moraxella catarrhalis							89	127		157
19	Budvicia aquatica				166				96		135
20	Buttiauxella agrestis			187	167				97		136

Table 8. Consensus PCR for detection of bacteria

- Continuation 1/6 -

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer H1 SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
21	Enterobacter agglomerans			188	168				98		
22	Erwinia carotovora			189	169				99		
23	Erwinia chrysanthemi			190	170				100		138
24	Escherichia coli			187	171				101		139
25	Escherichia hermannii			191	172				102		140
26	Escherichia vulneris			192	173				103, 165		141
27	Hafnia alvei			193	174				104		142
28	Klebsiella oxytoca			187	175				105, 165		143
29	Kluyvera cryoescens			187	175				106		144
30	Morganella morganii			194	176				107		145
31	Pantoea dispersa			187	177				108, 165		146
32	Pantoea			188	178				109, 165		147
33	Proteus mirabilis			195	179				110		
34	Proteus rettgeri			196	180				111		148
35	Providencia stuartii			197	181				112		149
36	Rahnella aquatilis			198	182				113, 164		149
37	Serratia ficaria								114, 164		150

Table 8. Consensus PCR for detection of bacteria

- Continuation 2/6 -

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer H1 SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
38	Serratia fonticola								115, 164		
39	Serratia marcescens								116, 164		
40	Serratia plymuthica								117, 164		
41	Serratia proteamaculans								118, 164		
42	Serratia rubidea								119, 164		
43	Yersinia enterolytica			199	184				120, 164		152
44	Yersinia pseudotuberculosis			200	185				121, 164		153
45	Aeromonas enteropelogenes								123		
46	Aeromonas hydrophila								124		
47	Cedecea davisae			201	186				125		
48	Stenotrophomonas multophila								129		159
49	Enterobacter agglomerans								137, 165		
50	Serratia				183						151
51	Citrobacter								202, 203		
52	Salmonella							204-210			
53	Pseudomonas stutzeri	213	252	289	326	361	403		434		488

Table 8. Consensus PCR for detection of bacteria

- Continuation 3/6 -

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
54	Thiobacillus ferrooxidans	214	253	290	327	362	404		435		489
55	Agrobacterium vitis	215	254	291	328	363			436		490
56	Adalia bipunctata	216	255	292	329	364			437		491
57	Amycolatopsis orientalis	217	256	293	330				438		
58	Brucella ovis	218	257	294	331	365			439		492
59	Bradyrizobium japonicum	219	258	295	331	366			440		493
60	Pseudomonas paucimobilis	220	259	296	332	367			441		494
61	Rhodobacter sphaeroides	221	260	297	333	368			442		495
62	Rickettsia prowazekii	222	261	298	333	369			443		496
63	Sphingomonas paucimobilis	223	262	299	334	370	405		525		499
64	Zymomonas mobilis	224	263	300	335	371			526		500
65	Alcaligenes	225	264	301	336	372	406		527		501
66	Pseudomonas cepacia	226	265	302	337		407		444		502
67	Ralstonia pickettii	227	266	303	338	373	408		445		503
68	Campylobacter jejuni	228	267	304	339	374	409		446		
69	Helicobacter pylori	229	268	305	340	375	410		447		504
70	Actinoplanes utahensis	230	269	306	341		411		448		

Table 8. Consensus PCR for detection of bacteria

- Continuation 4/6 -

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer H1 SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
71	Bacillus halodurans	231	270	307	342	376	412		449		505
72	Bacillus subtilis	232			343	377	413		450		506
73	Clostridium tyrobutyricum	233	271	308	344	378	414		451		507
74	Frankia	234	272	309	345	379	415		452		508
75	Microbispora bispora	235	273	310	346	380	416		453		509
76	Mycobacterium leprae	236	274	311	347	381	417		454		510
77	Mycobacterium smegmatis	237	275	312	348	382	418		455		511
78	Mycobacterium tuberculosis	238	276	313	349	383	419		456		512
79	Mycobacterium gallisepticum	239	277	314		384	420		457		
80	Propionibacterium freudenreich	240	278	315	350	385	421		458		
81	Rhodococcus erythropolis	241	279	316	351	386	422		459		513
82	Rhodococcus fascians	242				387	423		460		514
83	Staphylococcus aureus	243	280	317	352	388	424		461		515
84	Streptococcus faecalis	244	281	318	353	389	425		462		516
85	Streptomyces ambifaciens	245	282	319	354	390	426		463		517
86	Flavobacterium resinovorum	246	283	320	355	395	428		468		519
87	Sphingobacterium multivorans	247	284	321	356	396			469		520

Table 8. Consensus PCR for detection of bacteria

- Continuation 5/6 -

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer H1 SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
88	Synechococcus	248	285	322	357	397	429		470		521
89	Synechocystis	249	286	323	358	398	430		471		522
90	Borrelia burgdorferi	250	287	324	359	399			472, 428		523
91	Chlamydia trachomatis	251	288	325	360	400	431		473		524
92	Streptomyces galbus					391	426		464		
93	Streptomyces griseus					392	426		465		518
94	Streptomyces lividans					393	426		466		518
95	Streptomyces mashuensis					394	427		467		
96	Salmonella typhi						401		432		486
97	Buchnera aphidocola								433		487
98	Brucella orientalis								439		492
99	Brucella abortus								439		492
100	Azotobacter vinelandii								474		
101	Cowduria ruminantium								475, 530		
102	Mycobacterium intracellulare								476		
103	Mycobacterium lufu								477		
104	Mycobacterium simiae								478		

Table 8. Consensus PCR for detection of bacteria

- Continuation 6/6 -

No.	Taxonomic unit	Primer A1 SEQ ID	Primer B1 SEQ ID	Primer C1 SEQ ID	Primer D1 SEQ ID	Primer E1 SEQ ID	Primer F1 SEQ ID	Primer G1 SEQ ID	Primer H1 SEQ ID	Primer B2 SEQ ID	Primer A2 SEQ ID
105	Mycobacterium smegmatis								479		
106	Saccharomonospora azurea								480		
107	Saccharomonospora caesia								481		
108	Saccharomonospora cyanea								482		
109	Saccharomonospora glauca								483		
110	Saccharomonospora viridis								484		
111	Wolbachia pipientis								485		
112	Rickettsia bellii										497
113	Rickettsia rickettsii										498
114	Xanthomonas campestris								529		

Patent Claims

1. Nucleic acid molecules as a probe and/or a primer for detection of bacteria, selected from:

   a) nucleic acid molecules comprising at least one sequence with any of the SEQ ID NOs: 1 to 530 and/or a sequence from position 2667 to 2720, 2727 to 2776, 2777 to 2801, 2801 to 2832, 2857 to 2896, 2907 to 2931, 2983 to 2999 and/or 3000 to 3032 according to SEQ ID NO: 1; or nucleic acids which are homologous, analogous, or at least 70% identical with them;

   b) nucleic acid molecules which hybridize specifically with a nucleic acid according to a);

   c) nucleic acid molecules which exhibit 70%, preferably at least 90%, identity with a nucleic acid according to a) or b);

   d) nucleic acid molecules which are complementary to a nucleic acid according to any of a) to c).

2. Nucleic acid molecule according to Claim 1, **characterized in that** it is at least 10 nucleotides, preferably at least 14 nucleotides, long.

3. Nucleic acid molecule according to one of the preceding claims, **characterized in that** the nucleic acid molecule is modified such that up to 20% of the nucleotides in 10 successive nucleotides, particularly 1 or 2 nucleotides from the block of ten, are replaced by nucleotides which do not occur naturally in bacteria.

4. Nucleic acid molecule according to one of the preceding claims, **characterized in that** the nucleic acid molecule is modified or labeled so that it can generate a signal in analytical detection procedures which are known per se, with the modification selected from (i) radioactive groups, (ii) colored groups, (iii) fluorescent groups, (iv) groups for immobilization of a solid phase, and (v) groups

which allow a direct or indirect reaction, especially using antibodies, antigens, enzymes, and/or substances with affinity to enzymes or enzyme complexes.

5. Combination of at least 2 nucleic acid molecules, selected from

a) a combination of at least one DNA molecule which is shortened in comparison with the sequence SEQ ID NO: 1, position 2571 to 2906, and at least one DNA molecule which is shortened or not shortened in comparison with the transcribed spacer between the 23 S and 5 S genes corresponding to position 2907 to 2999 in SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

b) a combination of at least one DNA molecule which is shortened or not shortened in comparison with the transcribed spacer between the 23 S and 5 S genes, position 2907 to 2999 of SEQ ID NO: 1, and at least one DNA molecule which is shortened in comparison with the 5 S rDNA gene with the sequence between positions 3000 to 3112 of SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

c) a combination of at least one DNA molecule which is shortened or not shortened in comparison with the 23 S gene with the sequence from position 2907 to 2999 of SEQ ID NO: 1, and at least one shortened DNA molecule from the 5 S rDNA gene from position 3000 to 3112 of SEQ ID NO: 1, or DNA molecules with are homologous, analogous, or at least 75% identical with them;

d) a combination of at least one DNA molecule which is shortened in comparison with the 23 S gene with the sequence from position 2571 to 2906 of the SEQ ID NO: 1 and at least one shortened DNA molecule from the 5 S rDNA gene from position 3000 to 3112 of SEQ ID NO: 1, or DNA molecules which are homologous, analogous, or at least 75% identical with them;

e) a combination of 2 nucleic acid molecules according to Claim 1;

f) a combination containing at least one DNA molecule which hybridizes with a region hybridizing at least 100 nucleotides upstream from the 3' end of the 23 S rDNa, therefore within the 23 S rDNA;

wherein the combination according to any of a) to f) can also be a combined DNA molecule comprising at least 15 base pairs, for detection of bacteria or phylogenetic groups of bacteria, preferably of enterobacteria.

6. Kit, containing a nucleic acid molecule or a combination of nucleic acid molecules according to one of the preceding claims.

7. Method for detecting bacteria, preferably enterobacteria, in an analytical sample, comprising the step of bringing the analytical sample into contact with a nucleic acid or a combination of nucleic acids according to one of Claims 1 to 5, and detection of suitable hybrid nucleic acids comprising the added nucleic acid and bacterial nucleic acid.

8. Method for amplifying bacterial DNA of a multiplicity of different taxonomic units, especially genera and species, using primers according to Claims 1 – 5, in which

in a first amplification step the DNA for high taxonomic units such as classes, phyla or families is amplified with conserved primers, and, optionally, in at least one further amplification step (EN) parts of the first amplification fragment which are specific for genera, species or species can be multiplied with nested, increasingly variable primers, and, optionally, in a further step, the DNA fragments obtained by amplification which are specific for genera, species or species are detected by means of probes.

9. Method according to one of the preceding claims, **characterized in that** the process involves a PCR amplification of the nucleic acid to be detected.

10. Method according to one of the preceding claims, **characterized in that** the process involves a Southern Blot hybridization.

11. Use of a nucleic acid molecule according to one of Claims 1 to 5 to detect bacteria or bacterial nucleic acids.

12. Use of a nucleic acid molecule according to Claim 11, **characterized in that** the detection involves a polymerase chain reaction (PCR).

13. Use of a nucleic acid molecule according to Claim 11, **characterized in that** the detection involves a ligase chain reaction.

14. Use of a nucleic acid molecule according to Claim 11, **characterized in that** the detection involves an isothermal nucleic acid amplification.

15. Use of a nucleic acid molecule according to one of Claims 1 to 5 for the identification and/or characterization of bacteria.

16. Use of a nucleic acid molecule comprising a sequence with SEQ ID NO: 211 and/or 212, or derivatives derived from it, as defined in Claim 1a) to d) for the detection of any selected eubacteria or taxonomic units of the Eubacteria.

17. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 1 to 26, 34, 42, 54, 66, 78, 85, 135 to 153, 166 to 201, 92 to 121, 125 and/or 202 to 212 according to one of Claims 1 to 5 for the detection of the family of the Enterobacteriaceae or any selected bacterium of the family of the Enterobacteriaceae.

18. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 2 to 95, 135 to 201, 211 to 214, 252, 253, 289, 290, 326, 327, 361, 362, 401, 402, and/or 486 according to Claim 1 for the detection of the γ branch of the proteobacteria or any selected bacterium of the γ branch of the proteobacteria.

19. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 251, 288, 325, 326, 400, 431 and/or 524 according to Claim 1 for the detection of the

group of the Chlamydiales or Verrumicrobia or any selected bacterium from the group of the Chlamydiales or Verrumicrobia.

20. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 248, 285, 322, 357, 397, 429, 521, 249, 286, 323, 358, 398, 430 and/or 522 according to Claim 1 for the detection of the group of Cyanobacteria or any selected bacterium from the group of the Cyanobacteria.

21. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 395, 428, 519, 246, 283, 320, 355, 520, 247, 284, 321, 356, and/or 396 according to Claim 1 for the detection of the group of Cytophagales or the group of green sulfur bacteria or any selected bacterium from the group of Cytophagales or the group of green sulfur bacteria.

22. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 230 to 245, 269 to 282, 306-319, 341-354, 376-394, 411 to 427 and/or 505 to 518 according to Claim 1 for the detection of the group of Firmicutes or Gram-positive bacteria or any selected bacterium from the group of Firmicutes or Gram-positive bacteria.

23. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 250, 287, 324, 359,399, and/or 523 according to Claim 1 to detect the group of Spirochaetales or any selected bacterium from the group of Spirochaetales.

24. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 42, 96, 135 and/or 166 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the proteobacteria, **characterized in that** it detects

the genus Budvicica,
or any groups of species of the genus Budvicia,
or any strains of the genus Budvicia,

while excluding other closely and/or distantly related bacteria or microorganisms.

25. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 42, 114-119, 151, 164, and/or 183 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Serratia,
or any groups of species of the genus Serratia,
or any strains of the genus Serratia,

while excluding other closely and/or distantly related bacteria or microorganisms.

26. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 96, 125, 186 and/or 201 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Cedecea,
or any groups of species of the genus Cedecea,
or any strains of the genus Cedecea,

while excluding other closely and/or distantly related bacteria or microorganisms.

27. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 97, 136, 167 and/or 187 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Buttiauxella,
or any groups of species of the genus Buttiauxella,
or any strains of the genus Buttiauxella,

while excluding other closely and/or distantly related bacteria or microorganisms.

28. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 98, 137, 165, 168 and/or 188 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Enterobacter,
or any groups of species of the genus Enterobacter,
or any strains of the genus Enterobacter,

while excluding other closely and/or distantly related bacteria or microorganisms.

29. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 99, 100, 138, 139, 169, 170, 189 and/or 190 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Erwinia,
or any groups of species of the genus Erwinia,
or any strains of the genus Erwinia,

while excluding other closely and/or distantly related bacteria or microorganisms.

30. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 1, 1013, 140-142, 165, 171-173, 187, 191, and/or 192 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Escherichia,
or any groups of species of the genus Escherichia,
or any strains of the genus Escherichia,

while excluding other closely and/or distantly related bacteria or microorganisms.

31. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 104, 143, 174 and/or 193 according to Claim 1 for the detection of bacteria or

phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Hafnia,
or any groups of species of the genus Hafnia,
or any strains of the genus Hafnia,

while excluding other closely and/or distantly related bacteria or microorganisms.

32. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 105, 144, 165, 175 and/or 187 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Klebsiella,
or any groups of species of the genus Klebsiella,
or any strains of the genus Klebsiella,

while excluding other closely and/or distantly related bacteria or microorganisms.

33. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 107, 146, 176 and/or 194 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Morganella,
or any groups of species of the genus Morganella,
or any strains of the genus Morganella,

while excluding other closely and/or distantly related bacteria or microorganisms.

34. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 108, 109, 147, 165, 177, 178, 187 and/or 188 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Pantoea,
or any groups of species of the genus Pantoea,
or any strains of the genus Pantoea,

while excluding other closely and/or distantly related bacteria or microorganisms..

35. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 110, 111, 148, 149, 179, 180, 195 and/or 196 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Proteus,
or any groups of species of the genus Proteus,
or any strains of the genus Proteus,

while excluding other closely and/or distantly related bacteria or microorganisms.

36. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 121, 122, 152, 153, 164, 184, 185, 199 and/or 200 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Yersinia,
or any groups of species of the genus Yersinia,
or any strains of the genus Yersinia,

while excluding other closely and/or distantly related bacteria or microorganisms.

37. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 112, 149, 181, and/or 197 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Providencia,

or any groups of species of the genus Providencia,
or any strains of the genus Providencia,

while excluding other closely and/or distantly related bacteria or microorganisms.

38. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 113, 150, 164, 182 and/or 198 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Rahnella,
or any groups of species of the genus Rahnella,
or any strains of the genus Rahnella,

while excluding other closely and/or distantly related bacteria or microorganisms.

39. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 202 and/or 203 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Citrobacter,
or any groups of species of the genus Citrobacter,
or any strains of the genus Citrobacter,

while excluding other closely and/or distantly related bacteria or microorganisms.

40. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 204-210, 401, 432, and/or 486 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the genus Salmonella,
or any groups of species of the genus Salmonella,
or any strains of the genus Salmonella,

while excluding other closely and/or distantly related bacteria or microorganisms.

41. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 27, 35, 43, 55, 67, 79, 86, 122 and/or 154 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the group of fluorescent Pseudomonads of the γ group,

or any groups of genera, species or strains of the group of fluorescent Pseudomonads of the γ group,

or any genera, species, or strains of the group of fluorescent Pseudomonads of the γ group,

or the family of Moraxellaceae of the fluorescent Pseudomonads of the γ group,

or any genera, species or strains of the family of Moraxellaceae of the γ group,

or the genus Acinetobacter,

or any groups of species of the genus Acinetobacter,

or any strains of the genus Acinetobacter,

while excluding other closely and/or distantly related bacteria or microorganisms.

42. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 28, 36, 44, 56, 68, 80, 87, 123, 124 and/or 155 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that it** detects

the "Aeromonas group" of the γ group of the Proteobacteria,

or any groups of genera, species or strains of the "Aeromonas group" of the γ group,

or any genera, species or strains of the "Aeromonas group" of the γ group,

or the genus Aeromonas,

or any groups of species of the genus Aeromonas,

or any strains of the genus Aeromonas,

while excluding other closely and/or distantly related bacteria or microorganisms.

43. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 29, 37, 45, 57, 69, 81, 88, 126 and/or 156 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that it** detects

the family of Pasteurellaceae of the γ group,

or any groups of genera, species or strains of the family of Pasteurellaceae of the γ group,

or the genus Hemophilus,

or any groups of species of the genus Hemophilus,

or any strains of the genus Hemophilus

while excluding other closely and/or distantly related bacteria or microorganisms.

44. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 30, 38, 46, 58, 70, 82, 89, 127, and/or 157 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the group of fluorescent Pseudomonads of the γ group,

or any groups of genera, species or strains of the group of fluorescent Pseudomonads of the γ group,

or the family of Moraxellaceae of the fluorescent Pseudomonads of the γ group,

or any genera, species or strains of the family of Moraxellaceae of the γ group,

or the genus Moraxella,

or any groups of species of the genus Moraxella,

or any strains of the genus Moraxella,

while excluding other closely and/or distantly related bacteria or microorganisms.

45. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 31, 39, 47, 59, 71, 83, 128 and/or 158 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the family of Pasteurellaceae of the γ group,

or any genera, species or strains of the family of Pasteurellaceae of the γ group,

or the genus Pasteurella,

or any groups of species of the genus Pasteurella,

or any strains of the genus Pasteurella,

while excluding other closely and/or distantly related bacteria or microorganisms.

46. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 32, 40, 48, 60, 72, 84, 90, 129, and/or 159 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the Xanthomonas group of the γ group,

or any genera, species or strains of the Xanthomonas group of the γ group,

or the genus Stenotrophomonas,

or any groups of species of the genus Stenotrophomonas,

or any strains of the genus Stenotrophomonas,

while excluding other closely and/or distantly related bacteria or microorganisms.

47. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 33, 41, 50-53, 61-65, 73-77, 91-95, 130-134, 160-162 and/or 163 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the family of Vibrionaceae of the γ group,

or any genera, species or strains of the family of Vibrionaceae of the γ group,

or the genus Vibrio,

or any groups of species of the genus Vibrio,

or any strains of the genus Vibrio,

while excluding other closely and/or distantly related bacteria or microorganisms.

48. Use of the nucleic acid molecule SEQ ID NO: 474 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the family and/or members of the family of the Azotobacteriaceae,

or the genus Azotobacter,

or any groups of species of the genus Azotobacter,

or any strains of the genus Azotobacter

while excluding other closely and/or distantly related bacteria or microorganisms.

49. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 402, 433 and/or 487 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the genus Buchnera
or any groups of species of the genus Buchnera,
or any strains of the genus Buchnera

while excluding other closely and/or distantly related bacteria or microorganisms.

50. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 213, 252, 289, 326, 361, 403, 434 and/or 488 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the group of fluorescent Pseudomonads of the γ group of the Proteobacteria,
or the fluorescent genus Pseudomonas,
or any group of species of the genus Pseudomonas,
or any strains of the genus Pseudomonas

while excluding other closely and/or distantly related bacteria or microorganisms.

51. Use of a nucleic acid molecule comprising a sequence with SEQ ID NO: 529 according to Claims 1 – 10 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Xanthomonas group of the γ group of the Proteobacteria,
the genus Xanthomonas,
or any group of species of the genus Xanthomonas,
or any strains of the genus Xanthomonas

while excluding other closely and/or distantly related bacteria or microorganisms.

52. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 213, 252, 289, 326, 361, 403, 434 and/or 488 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria from the γ branch of the Proteobacteria, **characterized in that** it detects

the group of fluorescent Pseudomonads of the γ group,

or any groups of genera, species or strains of the group of fluorescent Pseudomonads of the γ group,

or any genera, species or strains of the group of fluorescent Pseudomonads of the γ group

or the genus Pseudomonas,

or the species Pseudomonas stutzeri,

or any strains of the genus Pseudomonas of the group of fluorescent Pseudomonads of the γ group

while excluding other closely and/or distantly related bacteria or microorganisms.

53. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 216, 255, 292, 329, 364, 437 and/or 491 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rickettsiales,

or the family Rickettsiaceae,

or the genus Adalia,

or any groups of species of the genus Adalia,

or any strains of the genus Adalia

while excluding other closely and/or distantly related bacteria or microorganisms.

54. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 215, 254, 291, 328, 363, 436 and/or 490 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the family Rhizobiaceae,

or the genus Agrobacterium,

or any groups of species of the genus Agrobacterium,

or any strains of the genus Agrobacterium

while excluding other closely and/or distantly related bacteria or microorganisms.

55. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 439 and/or 492 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rhizobiaceae group or Rhizobacteria,

the genus Brucella,

or any groups of species of the genus Agrobacterium,

or the species Brucella abortus

or any strains of the genus Brucella

while excluding other closely and/or distantly related bacteria or microorganisms.

56. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 218, 257, 294, 331, 365, 439 and/or 492 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rhizobiaceae group or Rhizobacteria,

or the genus Brucella,

or any groups of species of the genus Agrobacterium,

or the species Brucella ovis

or any strains of the genus Brucella

while excluding other closely and/or distantly related bacteria or microorganisms.

57. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 439 and/or 492 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rhizobiaceae group or Rhizobacteria,

or the genus Brucella,

or any groups of species of the genus Agrobacterium,

or the species Brucella orientalis

or any strains of the genus Brucella

while excluding other closely and/or distantly related bacteria or microorganisms.

58. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 219, 258, 295, 331, 366, 440 and/or 493 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Bradyrhizobium group,

or the genus Bradyrhizobium,

or any groups of species of the genus Bradyrhizobium,

or any strains of the genus Bradyrhizobium

while excluding other closely and/or distantly related bacteria or microorganisms.

59. Use of a nucleic acid molecule comprising a sequence with SEQ ID NO: 530 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rickettsiales,

or the family Rickettsiaceae,

or the Ehrlichieae,

or the genus Cowduria,

or any strains of the genus Cowduria

while excluding other closely and/or distantly related bacteria or microorganisms.

60. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 220, 259, 296, 332, 367, 441 and/or 494 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Zymomonas group of the α group of the Proteobacteria,

or the genus Sphingomonas,

or the species Pseudomonas paucimobilis,

or any strains of the genus Sphingomonas or of the species Pseudomonas paucimobilis

while excluding other closely and/or distantly related bacteria or microorganisms.

61. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 221, 260, 297, 333, 368, 442 and/or 495 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rhodobacter group of the α group of the Proteobacteria,

or the genus Rhodobacter,

or any strains of the genus Rhodobacter

while excluding other closely and/or distantly related bacteria or microorganisms.

62. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 222, 261, 298, 333, 369, 443, 496, 497 and/or 498 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rickettsiales,

or the Rickettsiaceae,

or the Rickettsieae,

or the genus Rickettsia,

or the species Rickettsia prowazekii or Rickettsia bellii or Rickettsia rickettsii,

or any strains of the genus Rickettsia

while excluding other closely and/or distantly related bacteria or microorganisms.

63. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 223, 262, 299, 334, 370, 405, 499 and/or 525 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Zymomonas group of the α group of the Proteobacteria,

or the genus Sphingomonas

or any strains of the genus Sphingomonas

while excluding other closely and/or distantly related bacteria or microorganisms.

64. Use of the nucleic acid molecule SEQ ID NO: 485 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Rickettsiales,

or the Rickettsiaceae,

or the Wolbachieae,

or the genus Wolbachia,

or any strains of the genus Wolbachia

while excluding other closely and/or distantly related bacteria or microorganisms.

65. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 224, 263, 300, 335, 371, 500 and/or 526 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Zymomonas group of the a group of the Proteobacteria,

or the genus Zymomonas,

or any strains of the genus Zymomonas

while excluding other closely and/or distantly related bacteria or microorganisms.

66. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 225, 264, 301, 336, 372, 406, 501 and/or 527 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Alcaligenaceae,

or the genus Alcaligenes,

or any strains of the genus Alcaligenes

while excluding other closely and/or distantly related bacteria or microorganisms.

67. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 226, 265, 301, 337, 407, 444 and/or 502 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Pseudomallei group of the Pseudomonads of the β group of the Proteobacteria,

or the genus Pseudomonas of the Pseudomallei group,

or any strains of the genus Pseudomonas of the Pseudomallei group

while excluding other closely and/or distantly related bacteria or microorganisms.

68. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 227, 266, 303, 338, 373, 408, 445 and/or 503 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Burkholderia group,

or the genus Ralstonia,

or any strains of the genus Ralstonia

while excluding other closely and/or distantly related bacteria or microorganisms.

69. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 228, 267, 304, 339, 374, 409 and/or 446 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Campylobacter group,

or the genus Campylobacter,

or the species Campylobacter jejuni,

or any strains of the genus Campylobacter

while excluding other closely and/or distantly related bacteria or microorganisms.

70. Use of a nucleic acid molecule comprising a sequence with SEQ ID NOs: 229, 268, 305, 340, 375, 410, 447 and/or 504 according to Claim 1 for the detection of bacteria or phylogenetic groups of bacteria, **characterized in that** it detects

the Helicobacter group,

or the genus Helicobacter,

or the species Helicobacter pylori,

or any strains of the genus Helicobacter

while excluding other closely and/or distantly related bacteria or microorganisms.

71. Nucleic acid molecule according to Claim 1, **characterized in that** the nucleic acid molecule according to alternative a)exhibits a sequence selected from SEQ ID NO: 211 and SEQ ID NO: 212.

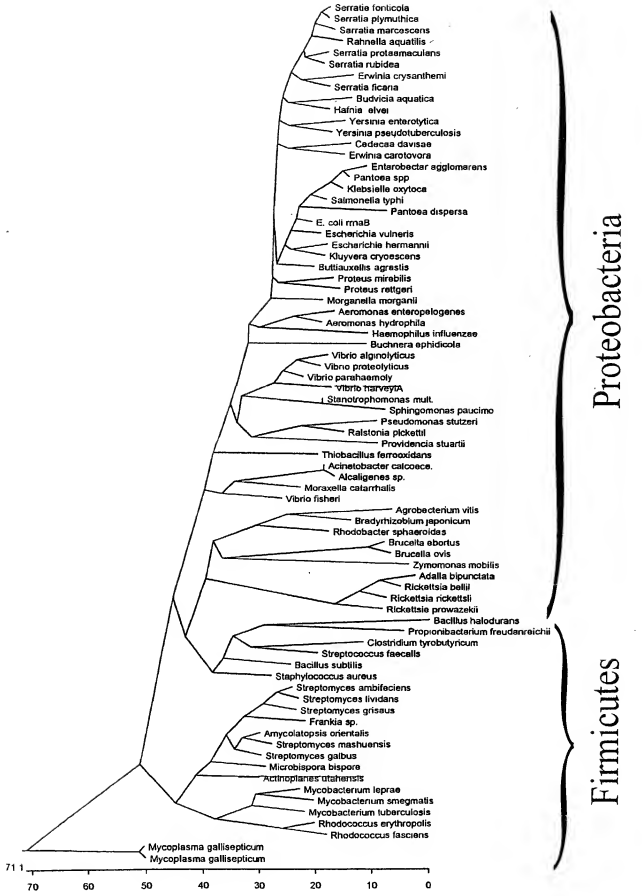
72. Combination according to Claim 5, **characterized in that** it contains at least one nucleic acid molecule with a sequence according to Claim 71.

73. Combination according to Claim 72, **characterized in that** it contains a nucleic acid molecule with a sequence according to SEQ ID NO: 211 and a nucleic acid molecule with a sequence according to SEQ ID NO: 212.

74. Kit, comprising a nucleic acid molecule according to Claim 71 and/or a combination according to Claim 72 or 73.

Summary

The present invention relates to nucleic acid molecules which allow the identification of bacteria or groups of bacteria. For detection, the region of the bacterial genome containing the 23 S / 5 S rRNA is used as the target sequence for the bacterial detection.


Serratia fonticola
Serratia plymuthica
Serratia marcescens
Rahnella aquatilis
Serratia proteamaculans
Serratia rubidea
Erwinia crysanthemi
Serratia ficaria
Budvicia aquatica
Hafnia alvei
Yersinia enterolytica
Yersinia pseudotuberculosis
Cedecea davisae
Erwinia carotovora
Enterobacter agglomerans
Pantoea spp
Klebsiella oxytoca
Salmonella typhi
Pantoea dispersa
E. coli rrnaB
Escherichia vulneris
Escherichia hermannii
Kluyvera cryoescens
Buttiauxella agrestis
Proteus mirabilis
Proteus rettgeri
Morganella morganii
Aeromonas enteropelogenes
Aeromonas hydrophila
Haemophilus influenzae
Buchnera aphidicola
Vibrio alginolyticus
Vibrio proteolyticus
Vibrio parahaemoly
Vibrio harveyiA
Stenotrophomonas mult.
Sphingomonas paucimo
Pseudomonas stutzeri
Ralstonia pickettii
Providencia stuartii
Thiobacillus ferrooxidans
Acinetobacter calcoace.
Alcaligenes sp.
Moraxella catarrhalis
Vibrio fisheri
Agrobacterium vitis
Bradyrhizobium japonicum
Rhodobacter sphaeroides
Brucella abortus
Brucella ovis
Zymomonas mobilis
Adalia bipunctata
Rickettsia bellii
Rickettsia rickettsii
Rickettsia prowazekii
Bacillus halodurans
Propionibacterium freudenreichii
Clostridium tyrobutyricum
Streptococcus faecalis
Bacillus subtilis
Staphylococcus aureus
Streptomyces ambifaciens
Streptomyces lividans
Streptomyces griseus
Frankia sp.
Amycolatopsis orientalis
Streptomyces mashuensis
Streptomyces galbus
Microbispora bispore
Actinoplanes utahensis
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Mycobacterium smegmatis
Mycobacterium tuberculosis
Rhodococcus erythropolis
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FIG. 1

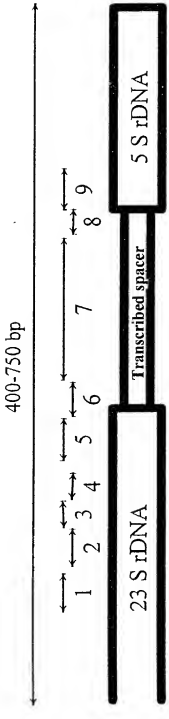

FIG. 2

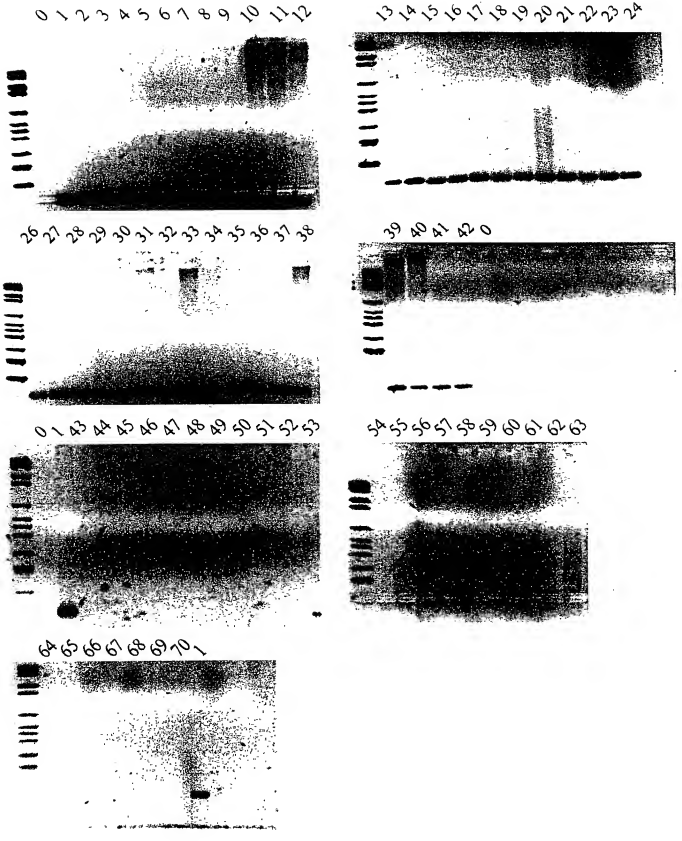
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FIG. 3

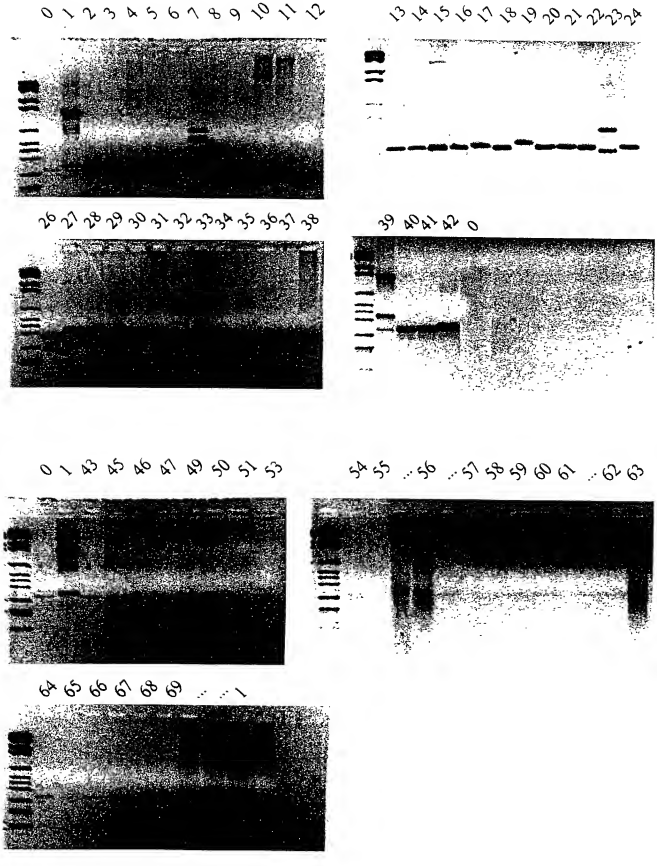
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FIG. 4

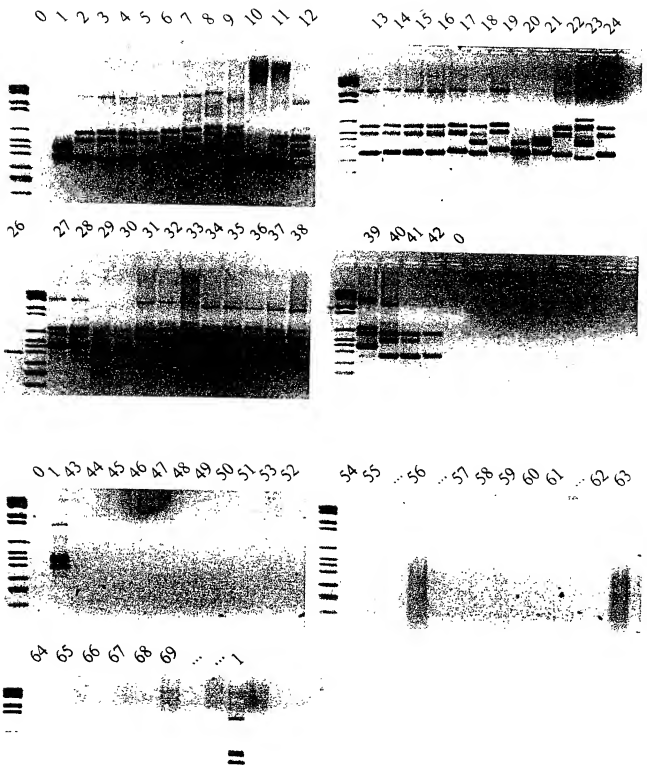
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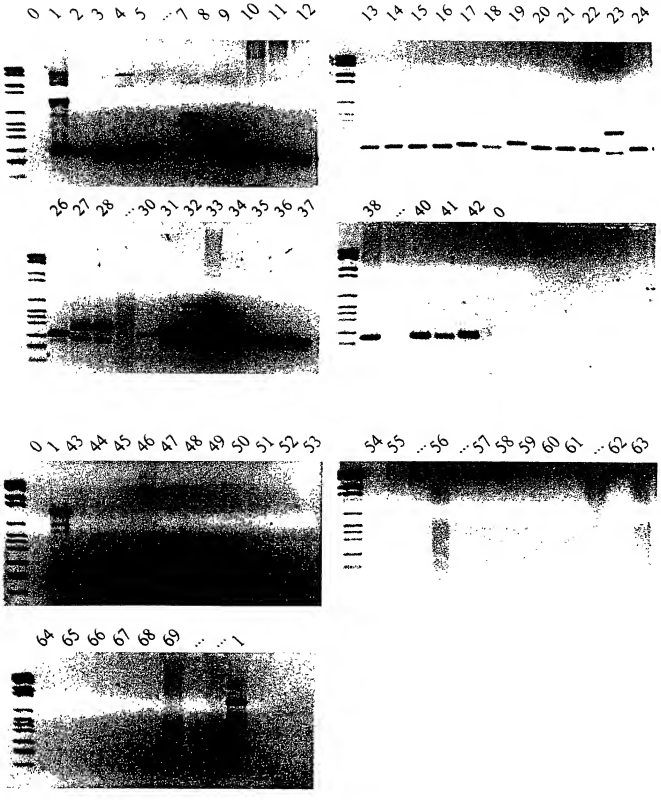
FIG. 5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64 65 66 67 68 69 1

FIG. 6

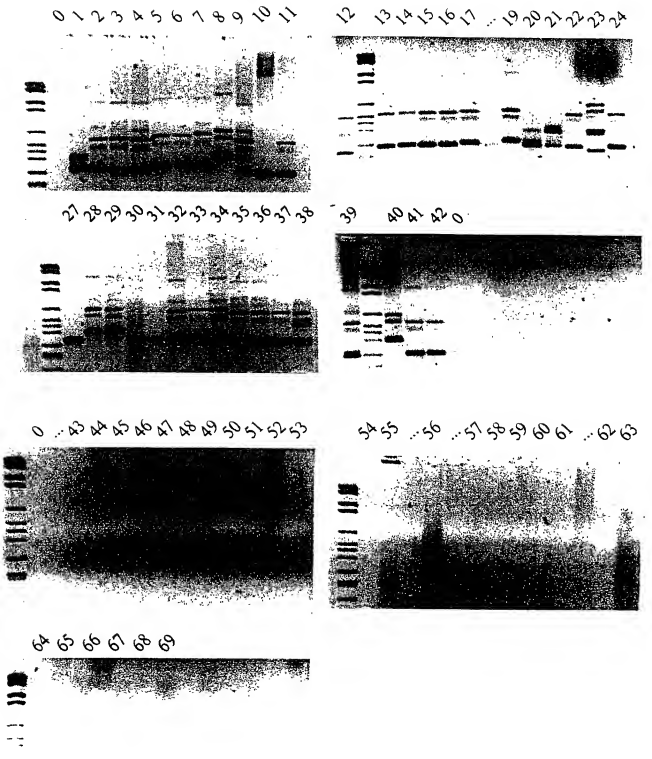
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FIG. 7

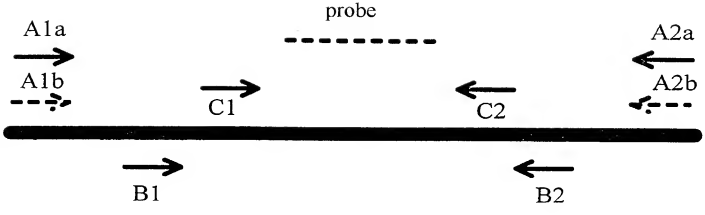

FIG. 8

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION AND POWER OF ATTORNEY

☐ Declaration Submitted with Initial Filing OR
☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name. I believe I am the original, first, and sole inventor *(if only one name is listed below)* or an original, first, and joint inventor *(if plural names are listed below)* of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NUCLEIC ACID MOLECULES FOR THE DETECTION OF BACTERIA AND PHYLOGENETIC UNITS OF BACTERIA

the specification of which:

- ☐ is attached hereto.
- ☒ was filed on March 22, 2002 as Application No. 10/088,966 and was amended on March 22, 2002 *(if applicable)*.
- ☐ was filed by Express Mail No. *as Application No. not known yet*, and was amended on *(if applicable)*.
- ☐ was filed on as PCT International Application No. PCT/ and was amended on *(if any)*.

I state that I have reviewed and understand the contents of the specification identified above, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I claim foreign priority benefits under 35 USC 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application(s) designating at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application(s) for patent, utility model, design registration, inventor's or plant breeder's rights certificate(s), or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter and having a filing date before that of the application(s) from which the benefit of priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Claimed YES	Priority Claimed NO	Certified Copy Attached? YES	Certified Copy Attached? NO
PCT/EP00/08813	WIPO	09/08/2000	☒	☐	☐	☒
199 45 916.9	Germany	09/24/1999	☒	☐	☐	☒
			☐	☐	☐	☐
			☐	☐	☐	☐

In re Appln. of Grabowski et al.
Attorney Docket No. 216180

As a named inventor, I hereby appoint Leydig, Voit & Mayer, Ltd. to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Customer Number 23460.

PATENT TRADEMARK OFFICE

I further direct that correspondence concerning this application be directed to Leydig, Voit & Mayer, Ltd.: Customer Number 23460.

PATENT TRADEMARK OFFICE

I declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100

Full name of **sole or first inventor:** Reiner GRABOWSKI

Inventor's signature R. Grabowski

Date 10.5.02

Country of Citizenship: Germany

Residence: Goettingen, Germany DEX
(city/state or country)

Post Office Address: Theodor-Heuss-Strasse 39, 37075 Goettingen, Germany
(complete mailing address)

In re Appln. of Grabowski et al.
Attorney Docket No. 216180

200

Full name of **second joint inventor, if any**: Kornelia ~~BERGHOF~~

Inventor's signature _____

Date 27.05.02

Country of Citizenship: Germany

Residence: Berlin, Germany
(city/state or country)

Post Office Address: Rhodelaender Weg 85, 12355 Berlin, Germany
(complete mailing address)

SEQUENCE LISTING

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<211> 42
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
derived from species of the genus Vibrio

<400> 41
tcgataaccg ctgaaagcat ctaagcggga agcgagcctt ga 42

<210> 42
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from genera of enterobacteria

<400> 42
agatgagtct tccctgggcc ttta 24

<210> 43
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived

from species of the genus Acinetobacter

<400> 43
agataagatt tccctaggac ttta 24

<210> 44
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Aeromonas

<400> 44
agatgagtca tccctgaccc cttg 24

<210> 45
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Haemophilus

<400> 45
agatgagtca tccctgactt t 21

<210> 46
<211> 13
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Moraxella

<400> 46
agataagatt tcc 13

<210> 47
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Pasteurella

<400> 47
agatgagatt tcccattacg c 21

<210> 48
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
derived from species of the genus
Stenotrophomonas

<400> 48
agatgagatt tcccggagcc ttg 23

<210> 49
<211> 24
<212> DNA
<213> Vibrio alginolyticus

<400> 49
agatgagttc tccctgatac ttta 24

<210> 50
<211> 13
<212> DNA
<213> Vibrio fisheri

<400> 50
agattagatt tcc 13

<210> 51
<211> 24
<212> DNA
<213> Vibrio harbeyi

<400> 51
agatgagtct tccctgggcc ttta 24

<210> 52
<211> 24
<212> DNA
<213> Vibrio parahaemolyticus

<400> 52
agatgagtct tccctgatac ttta 24

<210> 53
<211> 24
<212> DNA
<213> Vibrio proteolyticus

<400> 53
agatgagtct tccctggcac ttta 24

<210> 54
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived

from genera of enterobacteria

<400> 54
agggtcctga agggacgttg aagactacga cg 32

<210> 55
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Acinetobacter

<400> 55
tgtcctctaa agagccgttc gagactagga cg 32

<210> 56
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived from species of the genus Aeromonas

<400> 56
tgtcctctaa agagccgttc gagactagga cg 32

<210> 57
<211> 31
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived from species of the genus Haemophilus

<400> 57
aagtcagtaa gggttgttgt agactacgac g 31

<210> 58
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived from species of the genus Moraxella

<400> 58
ctaaagagcc gttgtagacg acgacg 26

<210> 59
<211> 31
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Pasteurella

<400> 59
aagtaagtaa gatccctcaa agacgatgag g 31

<210> 60
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
derived from species of the genus
Stenotrophomonas

<400> 60
agctccttga agggtcgttc gagaccagga cg 32

<210> 61
<211> 32
<212> DNA
<213> Vibrio alginolyticus

<400> 61
agtatcctaa agggttgtcg tagmtacgac gt 32

<210> 62
<211> 27
<212> DNA
<213> Vibrio fisheri

<400> 62
ctaaagagcc gttcaagact aggacgt 27

<210> 63
<211> 33
<212> DNA
<213> Vibrio harbeyi

<400> 63
agtatcctaa agggttgttc gagactagaa cgt 33

<210> 64
<211> 33
<212> DNA
<213> Vibrio parahaemolyticus

<400> 64
agtatcctaa agggttgttc gagactagaa cgt 33

<210> 65
<211> 33
<212> DNA

<213> Vibrio proteolyticus

<400> 65
agtgtcctga agggttgttc gagactagaa cgt 33

<210> 66
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from genera of enterobacteria

<400> 66
agcgatgcgt tgagctaacc agtactaatg acccgtgagg 40

<210> 67
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Acinetobacter

<400> 67
agtgatatgt gaagctgacc aatactaatt gctcgtgagg 40

<210> 68
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Aeromonas

<400> 68
ggcgacgtgt tgagctaacc catactaatt acccgtgagg 40

<210> 69
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Haemophilus

<400> 69
tgtgagtcat tgagctaacc aatactaatt gcccgagagg 40

<210> 70
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Moraxella

<400> 70
agtgatacat gtagctaacc aatactaatt gctcgtttgg 40

<210> 71
<211> 47
<212> DNA
<213> Pasteurella pneumotropica

<400> 71
tggcgacacg tgcagctgac gaatactaat cgatcgagga cttaacc 47

<210> 72
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
derived from species of the genus
Stenotrophomonas

<400> 72
agtaatgcat taagctaacc agtactaatt gcccgtacgg 40

<210> 73
<211> 40
<212> DNA
<213> Vibrio alginolyticus

<400> 73
tgtgaggcgt tgagctaacc tgtactaatt gcccgtgagg 40

<210> 74
<211> 40
<212> DNA
<213> Vibrio fisheri

<400> 74
agtgatgcgt gtagctaacc tgtactaatt gctcgtttgg 40

<210> 75
<211> 40
<212> DNA
<213> Vibrio harveyi

<400> 75
tgtgaggcgt tgagctaacc tgtactaatt gcccgtgagg 40

<210> 76
<211> 40
<212> DNA

<213> Vibrio paramaemolyticus

<400> 76
tgtgaggcat tgagctaact gatactaatt gcccgtgagg 40

<210> 77
<211> 40
<212> DNA
<213> Vibrio proteolyticus

<400> 77
tgtgaggcgt tgagctaacc tgtactaatt gcccgtgagg 40

<210> 78
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from genera of enterobacteria

<400> 78
acccgtgagg cttaacctta caacaccgaa 30

<210> 79
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Acinetobacter

<400> 79
gctcgtgagg cttgactata caacacccaa 30

<210> 80
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Aeromonas

<400> 80
acccgtgagg cttaaccata caacacccaa 30

<210> 81
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Haemophilus

<400> 81
gcccgagagg cttaactata caacgctcaa 30

<210> 82
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:derived
from species of the genus Moraxella

<400> 82
gctcgtttgg cttgaccata caacacccaa 30

<210> 83
<211> 33
<212> DNA
<213> Pasteurella pneumotropica

<400> 83
gctgacgaat actaatcgat cgaggactta acc 33

<210> 84
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Stenotrophomonas

<400> 84
gcccgtacgg cttgtcccta taaccttggt 30

<210> 85
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from genera of enterobacteria

<400> 85
caacaccgaa ggtgttttgg aggaatc 27

<210> 86
<211> 27
<212> DNA
<213> Acinetobacter calcoaceticus

<400> 86
caacacccaa gcagttgtat ataaagc 27

<210> 87
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Aeromonas

<400> 87
caacacccaa gaagtgttct aaggctt 27

<210> 88
<211> 27
<212> DNA
<213> Haemophilus influenzae

<400> 88
caacgctcaa gtgtttttgg gagctaa 27

<210> 89
<211> 27
<212> DNA
<213> Moraxella catarrhalis

<400> 89
caacacccaa gtggtttacc actgact 27

<210> 90
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence:
derived from species of the genus
Stenotrophomonas

<400> 90
taaccttggt agtccaaggt cgagtac 27

<210> 91
<211> 27
<212> DNA
<213> Vibrio alginolyticus

<400> 91
caacacccaa ggggttttga tggactc 27

<210> 92
<211> 27
<212> DNA
<213> Vibrio fisheri

<400> 92
caacacccaa gtggtttgta tcaagca 27

<210> 93
<211> 27
<212> DNA
<213> Vibrio harveyi

<400> 93
caacacccaa ggggttttga tggactc 27

<210> 94
<211> 27
<212> DNA
<213> Vibrio paramaemolyticus

<400> 94
caacacccaa ggggttttga tggactc 27

<210> 95
<211> 36
<212> DNA
<213> Vibrio proteolyticus

<400> 95
caacacccaa ggggttttga tggactcaat gaaaga 36

<210> 96
<211> 118
<212> DNA
<213> Budvicia aquatica

<400> 96
caacatccga ggtgttttaa ggaaagttga agagacgaaa gaataagtag aattccagct 60
tgaaccgaga ttgagttgat ggttgtgtga atgacacgac ggtcaataga cagaatat 118

<210> 97
<211> 111
<212> DNA
<213> Buttiauxella agrestis

<400> 97
caacaccgaa ggtgttttgg ttgagagact aagatattga attttcagct tgaaccgaga 60
ttttaagtcg atggttgtgt gaacagcatg acggttgatg aaacagaata t 111

<210> 98
<211> 193
<212> DNA
<213> Enterobacter aglomerans

<400> 98
caacgccgaa gatgttttgg cggattgaga agattttcag cattgattac agattttcgg 60
gaacgaaaga ttttacgctg aggcaaggcg gcaaatgaag taaaggaagg agcatacatg 120
agtatgtgac tgactttgcg aatgcagcca acgcagccac agtgaaaaag attcgtttct 180
ggcaacagaa ttt 193

<210> 99
<211> 123

<212> DNA
<213> Erwinia carotovora

<400> 99
caacaccgaa ggtgttttga gagtgactca aagagatgtt gataatcagc ttgttttagg 60
attggttctg atggttatgc gagagcgaaa gcgaagcatg acggttggga tgaaacagaa 120
ttt 123

<210> 100
<211> 101
<212> DNA
<213> Erwinia chrysanthemi

<400> 100
caacaccgaa ggtgttttag agagattggt ttgaattttc agtgaagttc cgagattggt 60
tctgatggct acggagtagc ggtcgggatg aaacaaaatt t 101

<210> 101
<211> 92
<212> DNA
<213> Escherichia coli

<400> 101
caacgccgaa gctgttttgg cggatgagag aagattttca gcctgataca gattaaatca 60
gaacgcagaa gcggtctgat aaaacagaat tt 92

<210> 102
<211> 104
<212> DNA
<213> Escherichia hermannii

<400> 102
caacgccaga gtggttttgg tgttgcggtg tgagagacga ttttcagctt gaccggatag 60
acatctgtgg cggcgcgcga gcacgcagca ggtgaacaga attt 104

<210> 103
<211> 92
<212> DNA
<213> Escherichia vulneris

<400> 103
caacgccgaa gatgttttgg cggatttgaa agacgatttt cagctgatac agattaagtc 60
tgccgcctga cggcgtcaga cagacagaat tt 92

<210> 104
<211> 119
<212> DNA
<213> Hafnia alvei

<400> 104
caacaccgaa ggtgttttaa gacgcagaga cgcgaaaaca caaagagtaa gcttgttgaa 60
cagattggtt tgtatggcta gctgtagaaa tacagaaagc ggtacaaata acagaatat 119

<210> 105
<211> 195
<212> DNA

<213> Klebsiella oxytoca

<400> 105
cgccgaagat gttttggcga tttgagaaga caacaatttc agcattgatt acagattttc 60
gggaacgaaa gattttacgc tgaggcaagg cggcaaatga aggaaaggaa ggagcatact 120
gaagtatgtg actgacttta cgaatgcagc caacgcagca tcggtgtaaa agattcgttt 180
ctgacaacag aattt 195

<210> 106
<211> 90
<212> DNA
<213> Kluyvera cryoescens

<400> 106
cgccaaagat gttttggtga aaagagacat caataatcag cttgatacag ataaattaac 60
tggccgaaag gcgggttaat aacagaattt 90

<210> 107
<211> 105
<212> DNA
<213> Morganella morganii

<400> 107
caccgaaggt gttttgagtt gagagacgat taaagagatt tttcagcaca gtgaagaggc 60
agaagtcatt cactgtgaaa gcttattttg gattgaaatg aattt 105

<210> 108
<211> 192
<212> DNA
<213> Pantoea dispersa

<400> 108
cgccagaggc gttttggtct gagagaccna aagaattttc agcattgttc accggattac 60
ntccagtgga ttttgtgctg tgacaaggcg gcacgcgaga cgacgggaag gagcatacac 120
gagtatgtga ctgagcggcg cgagcggggc aacgcagtca gagcgcaaaa gacgcggtnt 180
aaaacaaaat tt 192

<210> 109
<211> 190
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Pantoea

<400> 109
cgccgaagat gttttggcgg aatgagaaga ttttcagcat tgattacaga ttttcgggaa 60
cgaaagattt tacgctgagg caaggcggca aatgaagtaa aggaaggagc atacatgagt 120
atgtgactga ctttkcggat gcagccaacg cagccacagt gaaaaagatt cgtttctggc 180
aacagaattt 190

<210> 110
<211> 111
<212> DNA
<213> Proteus mirabilis

<400> 110
caacaccgaa agtgttttgt cagagagacg aaacgatgaa gtcagcttgt tcaanattga 60
attactggcg acttaccgaa aggaaagaag cgagtgatta aaaccgaatt t 111

<210> 111
<211> 139
<212> DNA
<213> Proteus rettgeri

<400> 111
caacaccgaa ggtgtttttag agagatagag ttgttttcaa gaaagagtga gaagccaaaa 60
ggtgaaggac acgcagcttg tttgagattg aggttctggt ttagtgaaga aaaaactaaa 120
cgggaacaaa acagaattt 139

<210> 112
<211> 137
<212> DNA
<213> Providencia stuartii

<400> 112
caacaccgaa ggtgtttttag agagacgaag agacgaattg ttgaagcgca cgagatagag 60
tggtgcgaaa aaatcagctt gttcaagatt gcagttctgg tttgcggtgt agacgcgaac 120
gggaacgaac cgaattt 137

<210> 113
<211> 135
<212> DNA
<213> Rahnella aquatilis

<400> 113
caacaccgaa ggtgtttttg atttgagaga cagactcgag agagtagatt ttcagcgaat 60
tgttccggta ttggttcgta tggcggcgtg tgatgagaaa ttatgacacg acgcggtatg 120
aatgaaacag aattt 135

<210> 114
<211> 100
<212> DNA
<213> Serratia ficaria

<400> 114
caacaccgaa ggtgttttag agagacgaat aattttcagc gaagttctta gattggttct 60
ggtggttacg cgagtaacgg ccaagaatga aacagaattt 100

<210> 115
<211> 106
<212> DNA
<213> Serratia fonticola

<400> 115
caacacccaa ggtgttttga agagattgaa gtagattttc agcgaagttc cgagattggt 60
ttcaatggcg acacgagagt gaagcggttg aaatgaaaca gaattt 106

<210> 116
<211> 97
<212> DNA
<213> Serratia marcescens

<400> 116
caacaccgaa ggtgtttta gagagatttt cagcgaagtt ccgagattgg ttctgatggc 60
gacacgaaag tgaagcggtt ggaatgaaac agaattt 97

<210> 117
<211> 99
<212> DNA
<213> Serratia plymuthica

<400> 117
caacaccgaa ggtgttttag agagattaca gtagattttc agcgacgttc cgagattggt 60
ttcaatggcc caaaaggcgg ttggaatgaa acagaattt 99

<210> 118
<211> 100
<212> DNA
<213> Serratia proteamaculans

<400> 118
caacaccaaa ggtgtttag agagattgta gagattttca gcgagttccg agattggttt 60
caatggctgc gagagtagcg gttggaatga aacanaattt 100

<210> 119
<211> 101
<212> DNA
<213> Serratia rubidea

<400> 119
caacaccgaa ggtgttttag agagattggt ttgaattttc agtgaagttc cgagattggt 60
tctgatggct acggagtagc ggtcgggatg aaacagaatt t 101

<210> 120
<211> 116
<212> DNA
<213> Yersinia enterolytica

<400> 120
caacaccaaa ggtgttttgt atttgagaga tagatattga ttttcagcga atgttccgag 60
attgggctgg ctggctgtgt gaaagattgc atagcgggtt agtttagaca gaattt 116

<210> 121
<211> 104
<212> DNA
<213> Yersinia pseudotuberculosis

<400> 121
caacaccgaa gtcttgaatt gagagagatt ttcagcgtcg ttccgagatt ggattgactg 60
gcgtcacaag cgctgtttgt gtgcgggtta attaaaacag attt 104

<210> 122
<211> 179
<212> DNA
<213> Acinetobacter calcoaceticus

<400> 122

caacacccaa gcagttgtat ataaagcatc aatcgattca ttaatatgca aagcaacttg 60
atttagttat acgcttagct aaaatgaaca aaatatagta agactcaatc agcccatctg 120
taaagatttg gaaaacgcat cggcaaccaa taagaccaat gcaagtatcc ataccagtt 179

<210> 123
<211> 118
<212> DNA
<213> Aeromonas enteropelogenes

<400> 123
caacacccaa gaagtgtttn tggtgcttgt agcgaatgaa cgaactacgc attcagtgat 60
aacgacaagc cacgagcaac atcgttattc acgtcagctt tccaagattg aagatttt 118

<210> 124
<211> 81
<212> DNA
<213> Aeromonas hydrophila

<400> 124
caacacccaa gaagtgttct aaggcttgta gcagataccg agaacgaaca acaaaatcag 60
ctttctcaga ttgaagaatt t 81

<210> 125
<211> 96
<212> DNA
<213> Cedecea davisae

<400> 125
caacaccaaa ggtgttttgc gagacgcaat tttaattttc agcgaagttc aggattagac 60
tgatggtcac aaagtgacgg tcagtaaaca gaattt 96

<210> 126
<211> 217
<212> DNA
<213> Haemophilus influenzae

<400> 126
caacgctcaa gtgtttttgg gagctaagtg aagtaagaga tgaaaagcga agcaaataaa 60
agcagagcga aagagaagta aaagactaaa caaagaaaag taaatataga agacttaata 120
gaaagaaaat cggattcagc ttgtgaccaa taagaacgag tgaaaggtag aggaaagact 180
gagtaacgag agataaaaga gacgagagat aaaagag 217

<210> 127
<211> 90
<212> DNA
<213> Moraxella catarrhalis

<400> 127
caacacccaa gtggtttacc actgactgtg ttgattggta atatataaga tgaaccttaa 60
tcttgatttg gtaataaaca gactcataca 90

<210> 128
<211> 134
<212> DNA
<213> Pasteurella pneumotropica

<400> 128
cgaggactta accaaatttg tttatcgtaa caatgtcgtt tatccagttt tgaaagaata 60
aatttttatt aaataactct tgcattattc tacagagttg ttataataaa acatgtcctt 120
caaaagtatt caag 134

<210> 129
<211> 141
<212> DNA
<213> Stenotrophomonas multophila

<400> 129
taaccttggt agtccaaggt cgagtacaac tgctcgatac aaaagctaca acccnactta 60
cttcttccag attcatggcc acgctgaaca aagcgtaggg tgggcggctg tnccgcccac 120
gcgtaactca agcgtagcca g 141

<210> 130
<211> 100
<212> DNA
<213> Vibrio alginolyticus

<400> 130
caacacccaa ggggttttga tggactcaat gaaagaacat tgaatgtgta agaacgagaa 60
ttaaaaaaca gctttccaga ttaaagaatt tgcttggcga 100

<210> 131
<211> 122
<212> DNA
<213> Vibrio fisheri

<400> 131
caacacccaa gtggtttgta tcaagcatta tatcgatatc accgttatcc ttgattcagt 60
taggataagt gatacttaag tcattaagta aaacaaacac agactcatat ctaacccct 120
tt 122

<210> 132
<211> 122
<212> DNA
<213> Vibrio harveyi

<400> 132
caacacccaa gtggtttgta tcaagcatta tatcgatatc accgttatcc ttgattcagt 60
taggataagt gatacttaag tcattaagta aaacaaacac agactcatat ctaacccct 120
tt 122

<210> 133
<211> 89
<212> DNA
<213> Vibrio paramaemolyticus

<400> 133
caacacccaa ggggttttga tggactcgaa gcaagaacag aattgaatgt gtagagaaca 60
caaaaacagc tttccgaatt aaagaattt 89

<210> 134
<211> 169
<212> DNA

<213> Vibrio proteolyticus

<400> 134
caacacccaa ggggttttga tggactcaat gaaagaacat tgaatgtgta agaacgagaa 60
ttaaaaaaca gctttccgaa tttaggaatt gaatttatta acgacatcca tgtcgttaac 120
ccttcgggcc gcactgaagt gcgttaaatt ttgttccaga caaaatttt 169

<210> 135
<211> 33
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from genera of enterobacteria

<400> 135
gcctggcggc actagcgcgg tggtcccacc tga 33

<210> 136
<211> 33
<212> DNA
<213> Buttiauxella agrestis

<400> 136
gcctggcggc agtagcgcgg tggtcccacc tga 33

<210> 137
<211> 33
<212> DNA
<213> Enterobacter agglomerans

<400> 137
gcctggcggc tttagcgcgg tggtcccacc tga 33

<210> 138
<211> 33
<212> DNA
<213> Erwinia carotovora

<400> 138
gcctggcggc gatagcgcgg tggtcccacc tga 33

<210> 139
<211> 33
<212> DNA
<213> Erwinia chrysanthemi

<400> 139
gcctggcggc ggtagcgcgg tggtcccacc tga 33

<210> 140
<211> 33
<212> DNA
<213> Escherichia coli

<400> 140
gcctggcggc agtagcgcgg tggtcccacc tga 33

<210> 141
<211> 33
<212> DNA
<213> Escherichia hermannii

<400> 141
gcctggcggc aagagcgcgg tggtcccacc tga 33

<210> 142
<211> 33
<212> DNA
<213> Escherichia vulneris

<400> 142
gcctggcggc actagcgcgg tggtcccacc tga 33

<210> 143
<211> 33
<212> DNA
<213> Hafnia alvei

<400> 143
gcctggcggc gatagcgcgg tggtcccacc tga 33

<210> 144
<211> 32
<212> DNA
<213> Klebsiella oxytoca

<400> 144
gcctggcggc actagcgcgg tggtccacct ga 32

<210> 145
<211> 33
<212> DNA
<213> Kluyvera cryoescens

<400> 145
gcctggcggc aacagcgcgg tggtcccacc tga 33

<210> 146
<211> 33
<212> DNA
<213> Morganella morganii

<400> 146
gcctggcggc cgtagcgcgg tggtcccacc tga 33

<210> 147
<211> 31
<212> DNA
<213> Pantoea dispersa

<400> 147
gcctggcggc aacagccgcg gtggtcccac c 31

<210> 148
<211> 33
<212> DNA
<213> Proteus mirabilis

<400> 148
gcttggtggc catagcgcgg tggtcccacc tga 33

<210> 149
<211> 33
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genera Proteus, Providencia

<400> 149
gtctggcggc aatagcacgg tggtcccacc tga 33

<210> 150
<211> 33
<212> DNA
<213> Rahnella aquatilis

<400> 150
gcctggcggc agtagcgcgg tggtcccacc tga 33

<210> 151
<211> 33
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Serratia

<400> 151
gcctggcggc aatagcgcgg tggtcccacc tga 33

<210> 152
<211> 33
<212> DNA
<213> Yersinia enterolytica

<400> 152
gcctggcggc catagcgcgg tggacccacc tga 33

<210> 153
<211> 33
<212> DNA
<213> Yersinia pseudotuberculosis

<400> 153
gtctggcggc catagcgcgg tggtcycacc tga 33

<210> 154
<211> 51
<212> DNA
<213> Acinetobacter calcoaceticus

<400> 154
aagtatccat accagttgtg ctggcgacca tagcaagagt gaaccacctg a 51

<210> 155
<211> 33
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Aeromonas

<400> 155
gcctggcggc catagcgccg tggaaccacc tga 33

<210> 156
<211> 51
<212> DNA
<213> Haemophilus influenzae

<400> 156
aaaagacgag ttatcaaaga attatcctgg cggcgatagt gcggtggacc c 51

<210> 157
<211> 54
<212> DNA
<213> Moraxella catarrhalis

<400> 157
acagcgttgt taatcctttt acgctgacga caatagcaag atggaaccac ctga 54

<210> 158
<211> 43
<212> DNA
<213> Pasteurella pneumotropica

<400> 158
tctagtgatg atggcgaaga ggtcacaccc gttcccatac cga 43

<210> 159
<211> 54
<212> DNA
<213> Stenotrophomonas multophila

<400> 159
acaagtcaaa gcctgatgac catagcaagt cggtcccacc ccttcccatc ccga 54

<210> 160
<211> 33
<212> DNA
<213> Vibrio alginolyticus

<400> 160
gcttggcgac catagcgttt tggacccacc tga 33

<210> 161
<211> 51
<212> DNA
<213> Vibrio fisheri

<400> 161
ctcatatcta acccccttttg ctgacgacaa tagcacgatg gcaccacctg a 51

<210> 162
<211> 45
<212> DNA
<213> Vibrio harveyi

<400> 162
gcttggcgac catagcgatt tggacccacc tgacttccat tccga 45

<210> 163
<211> 33
<212> DNA
<213> Vibrio proteolyticus

<400> 163
gcttggcgac catagcgttt tggacccacc tga 33

<210> 164
<211> 37
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genera Rahnella, Serratia, Yersinia

<400> 164
agattttcag cgaagttccg agattggttt caatggc 37

<210> 165
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genera Enterobacter, Escherichia, Klebsiella, Pantoea

<400> 165

ggaaggagca taciiiagtat 18

<210> 166
<211> 32
<212> DNA
<213> Budvicia aquatica

<400> 166
aggtccctga aggaacgttt gagactaaga cg 32

<210> 167
<211> 32
<212> DNA
<213> Buttiauxella agrestis

<400> 167
agggtcctga aggaacgttg aagactacga cg 32

<210> 168
<211> 32
<212> DNA
<213> Enterobacter agglomerans

<400> 168
aggacactaa aggaacgttg aagacgacga cg 32

<210> 169
<211> 32
<212> DNA
<213> Erwinia carotovora

<400> 169
atgcccctga agggccgttg aagactacga cg 32

<210> 170
<211> 32
<212> DNA
<213> Erwinia chrysanthemi

<400> 170
aggcccctga agggacgttt aagacgaaga cg 32

<210> 171
<211> 29
<212> DNA
<213> Escherichia coli

<400> 171
agggtcctga aggaacgttg aagacgacg 29

<210> 172
<211> 32
<212> DNA
<213> Escherichia hermannii

<400> 172
agagtcctga aggaacgttg aagacgacga cg 32

<210> 173
<211> 32
<212> DNA
<213> Escherichia vulneris

<400> 173
agtctcctga aggaacgttg aagacgacga cg 32

<210> 174
<211> 32
<212> DNA
<213> Hafnia alvei

<400> 174
agtctcctaa aggaacgttt aagactaaga cg 32

<210> 175
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genera Klebsiella, Kuyvera

<400> 175
agggtcctga aggaacgttg aagacgacga cg 32

<210> 176
<211> 32
<212> DNA
<213> Morganella morganii

<400> 176
agggtcctga aggaacgttt gagactaaga cg 32

<210> 177
<211> 32
<212> DNA
<213> Pantoea dispersa

<400> 177
agggtcctga agggacgctg aagacgacga cg 32

<210> 178
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Pantoea

<400> 178
aggacactaa aggaacgtta aagacgatga cg 32

<210> 179
<211> 32
<212> DNA
<213> Proteus mirabilis

<400> 179
agtgacctaa aggaacgttt aagactaaga cg 32

<210> 180
<211> 32
<212> DNA
<213> Proteus rettgeri

<400> 180
agggtcctaa aggaacgttt aagactaaga cg 32

<210> 181
<211> 32
<212> DNA
<213> Providencia stuartii

<400> 181
agggtcctaa aggaacgttt aagacgaaga cg 32

<210> 182
<211> 32
<212> DNA
<213> Rahnella aquatilis

<400> 182
agccacctga agggacgttt aagactaaga cg 32

<210> 183
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Serratia

<400> 183
aggcccctga aggaacgttt aagactaaga cg 32

<210> 184
<211> 32
<212> DNA
<213> Yersinia enterolytica

<400> 184
agccccctga aggaacgtta aagactatga cg 32

<210> 185
<211> 32
<212> DNA
<213> Yersinia pseudotuberculosis

<400> 185
agccccctga gggaacgtta aagactatga cg 32

<210> 186
<211> 32
<212> DNA
<213> Cedecea davisae

<400> 186
agaccctga agggacgttg aagactacga cg 32

<210> 187
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genera Buttiauxella, Escherichia, Klebsiella, Kluyvera, Pantoea

<400> 187
agatgagttc tccctgaccc ttta 24

<210> 188
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genera Enterobacter, Pantoea

<400> 188
agatgagttc tcccttgtcc ttta 24

<210> 189
<211> 24
<212> DNA
<213> Erwinia carotovora

<400> 189
agatgagtct tccctgggca ccag 24

<210> 190
<211> 24
<212> DNA
<213> Erwinia chrysanthemi

<400> 190
agatgagtct tccctgggcc cttg 24

<210> 191
<211> 24
<212> DNA
<213> Escherichia hermannii

<400> 191
agatgagttc tccctgactc cttg 24

<210> 192
<211> 24
<212> DNA
<213> Escherichia vulneris

<400> 192
agatgagttc tccctgagac ttta 24

<210> 193
<211> 24
<212> DNA
<213> Hafnia alvei

<400> 193
agatgagtct tccctgagac cttg 24

<210> 194
<211> 24
<212> DNA
<213> Morganella morganii

<400> 194
agatgagtct tccctgaccc ttta 24

<210> 195
<211> 24
<212> DNA
<213> Proteus mirabilis

<400> 195
agatgagtct tccctgtcac ttta 24

<210> 196
<211> 24
<212> DNA
<213> Proteus rettgeri

<400> 196
agatgagtct tccctgaccc ttta 24

<210> 197
<211> 24
<212> DNA
<213> Providencia stuartii

<400> 197
agatgagtct tccctgactc ttta 24

<210> 198
<211> 24
<212> DNA
<213> Rahnella aquatilis

<400> 198
agatgagtct tccctgtggc ttta 24

<210> 199
<211> 24
<212> DNA
<213> Yersinia enterolytica

<400> 199
agatgagtct tccctggggc ttta 24

<210> 200
<211> 24
<212> DNA
<213> Yersinia pseudotuberculosis

<400> 200
agatgagtct tccctggggc ttaa 24

<210> 201
<211> 24
<212> DNA
<213> Cedecea davisae

<400> 201
agatgaattc tccctgggtc cttg 24

<210> 202
<211> 199
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Citrobacter

<400> 202
caacgccgaa gatgttttgg cggaattgag aagattttca gcattgattc agagtccgaa 60
ggattttgcg ctgagacaag gcggcawccc caccacggaa ggagcataca aaagtatgtg 120
actgaggttc gcaagcgcag ccaacgcagt atcagcacaa aagacacagg acagagcaca 180
aagaatttct ggcggccgt 199

<210> 203
<211> 199
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Citrobacter

<400> 203
caacgccgaa gatgttttgg cggattgaga agatttcag tattgattac agattttgcg 60
aaaacgaaag attttacgct gaggcaaggc ggcaagtgaa gcgacggaag kggcatacaa 120
aagtatgtga ctgaggttcg caggcgcagc caacgcagca tcagtggaaa agattcgttt 180
taagagcaca aagaatttc 199

<210> 204
<211> 199
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 204
caacsccsaa gatgttttgg csgatsagag argattttca gcactgattc ckgattttcg 60
vgaacgaaag attttacgct gaggcaaggc rgcaavcgaa ggaaaggaag gagcatactg 120
aagtatgtga ctgactttac gagcgcagcc aacgctagca tcsgtgtaaa agattcgttt 180
ctggcaacag aatttcctg 199

<210> 205
<211> 201
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 205
caacgccgaa gctgttttgg cggatranaa sacgaacaat tttcagcact gattcagagt 60
tgagtacgca ataatttgcg cagcagcaag gcggcaagcg aaggaaagga aggagcatac 120
agaagtatgt gactgacttt acgagcgcag ccaacgccgc tgatgcgata aagaattgcg 180
tacagagcac aaaagaatat t 201

<210> 206
<211> 193
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 206
caacgccgaa gatgttttgg csgttgagaa gacgattttc agcagtgatt ccgrgttgag 60
trcgcmrtaa tttkcgcmgc wgcarggcgg cargcgaagg arrggaggga gcatccwgaa 120
gtatktgact gagttttcgr gcgcwggcam cgccgctgat gcgataaaga attgcgtach 180
gmgcacamag aat 193

<210> 207
<211> 199
<212> DNA
<213> Artificial sequence

<220>

<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 207
caacgccgaa gatgttttgg cggattgaga gacgattttc agcactgatt ccggattttc 60
gggaacgaaa gattttacgc tgaggcaagg cggcaaatgr aggaaaggaa ggagcatact 120
gaagtatgtg actgactttt cgaatgcagc cgacgcagca tcggtgtaaa agattcgttt 180
ccggcaacag aattgtcct 199

<210> 208
<211> 189
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 208
caacgccgaa gatgttttgg cggatgagag acgattttca gcactgattc agagttgagt 60
acgcaataat ttgcgcagca gcaaggcggc aagcgaagga aaggaaggag catacagaag 120
tatgtgactg agtttacgag cgcaggcaac gccgctgatg cgataaagaa ttgcgtactg 180
agcataaaa 189

<210> 209
<211> 196
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 209
caacgccgaa gatgttttgg cggattgaga agacaacaat tttcagcyca gattcagagt 60
ccgaaggatt ttacgctgag acaaggcggc aaacgcagcs mcsgaaggas cmycacagaa 120
gtatgtgact gacgctcgca agagcagcca acgccgtatc agtgtaaaag acacaggacg 180
grgcacaaag aaattt 196

<210> 210
<211> 77
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Salmonella

<400> 210
gagagacgat tttcagcact gattccggat tttcgggaac gaaagataaa agattcgttt 60
ccggcaacag aatttcc 77

<210> 211
<211> 24
<212> DNA
<213> Artificial sequence

<220>

<223> Description of the artificial sequence: derived
from species and genera of eubacteria

<400> 211
ggtacgcgag ctgggtttag aacg 24

<210> 212
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species and genera of eubacteria

<400> 212
gbgagagtag gdmayygcc 19

<210> 213
<211> 54
<212> DNA
<213> Pseudomonas stutzeri

<400> 213
ccggagtgga cgaacctctg gtgttccggt tgtcacgcca gtggcattgc cggg 54

<210> 214
<211> 53
<212> DNA
<213> Thiobacilluc ferrooxidans

<400> 214
ccggagtgga cgtactctgg tgttccggtt gttctgccaa gggcattgcc ggg 53

<210> 215
<211> 54
<212> DNA
<213> Agrobacterium vitis

<400> 215
ccgggatgga catatctctg gtggacctgt tgtcgtgcca acggcatagc aggg 54

<210> 216
<211> 54
<212> DNA
<213> Adalia bipunctata

<400> 216
ccgaggtgga cgtacctctg gtggaccagt tgtcatgcca atggcacagc tggg 54

<210> 217
<211> 54
<212> DNA
<213> Amycolatopsis orientalis

<400> 217

ccgggacgga cgaacctctg gtgtgccagt tgtcctgcca agggcatggc tggt 54

<210> 218
<211> 54
<212> DNA
<213> Brucella ovis

<400> 218
ccgggatgga cgtatctntg gtggacctgt tgtggcgcca gccgcatagc aggg 54

<210> 219
<211> 54
<212> DNA
<213> Bradyrhizobium japonicum

<400> 219
ccggggtgaa cgtacctctg gtggagctgt tgtcgcgcca gcggcagtgc agca 54

<210> 220
<211> 54
<212> DNA
<213> Pseudomonas paucimobilis

<400> 220
ccgggatgga cgcaccgctg gtgtaccagt tgttctgcca agggcatcgc tggg 54

<210> 221
<211> 54
<212> DNA
<213> Rhodobacter sphaeroides

<400> 221
ccgggatgga cgcaccgctg gtgtaccagt tgttctgcca agggcatcgc tggg 54

<210> 222
<211> 57
<212> DNA
<213> Rickettsia prowazekii

<400> 222
ccgaggtgga cgtacccctg gtggaccagt tgtcgtgcca acggcaagct gggtagc 57

<210> 223
<211> 54
<212> DNA
<213> Sphingomonas paucimobilis

<400> 223
ccggagtgga cgaacctctg gtgtaccggt tgtcacgcca gtggcattgc cggg 54

<210> 224
<211> 54
<212> DNA
<213> Zymomonas mobilis

<400> 224
ccggggtgaa catgcctctg gtggacctgt cgtggcgcca gccgcgcagc aggg 54

<210> 225
<211> 54
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Alcaligenes

<400> 225
ccagagtgga cgaacctctg gtgtaccggt tgtgacgcca gtcgcatcgc cggg 54

<210> 226
<211> 53
<212> DNA
<213> Pseudomonas cepacia

<400> 226
ccgggacgac gaacctctgg tgtgtcagtt gtactgccaa gtgcaccgct gat 53

<210> 227
<211> 54
<212> DNA
<213> Ralstonia pickettii

<400> 227
ccggagtgga cgaacctctg gtgttccggt tgtcacgcca gtggcattgc cggg 54

<210> 228
<211> 54
<212> DNA
<213> Campylobacter jejuni

<400> 228
ccgggttgaa caaaccactg gtgtagctgt tgttctgcca agagcatcgc agcg 54

<210> 229
<211> 53
<212> DNA
<213> Helicobacter pylori

<400> 229
ccgggatgga cgtgtcactg gtgcaccagt tgtctgccaa gagcatcgct ggg 53

<210> 230
<211> 53
<212> DNA
<213> Actinoplanes utahensis

<400> 230
ccgggacgga cgaacctctg gtgtgccagt tgttctgcca agagcacggc tgg 53

<210> 231
<211> 54
<212> DNA
<213> Bacillus halodurans

<400> 231
ccgggatgga cacaccgctg gtgtaccagt tgttccgcca ggagcatcgc tggg 54

<210> 232
<211> 54
<212> DNA
<213> Bacillus subtilis

<400> 232
ccgggatgga cgcaccgctg gtgtaccagt tgttctgcca agggcatcgc tggg 54

<210> 233
<211> 54
<212> DNA
<213> Clostridium tyrobutyricum

<400> 233
ccgggatgga ctgacctctg gtgtaccagt tgttccgcca ggagcatggc tggg 54

<210> 234
<211> 54
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Frankia

<400> 234
ccgggacgga cgaacctctg gtgtgccagt tgttctgcca agggcatggc tggt 54

<210> 235
<211> 54
<212> DNA
<213> Microbispora bispora

<400> 235
ccggaacgga cgaacctctg gtgtgccagt tgtgccgcca ggtgcacggc tggt 54

<210> 236
<211> 54
<212> DNA
<213> Mycobacterium leprae

<400> 236
ccgggacgga cgaacctctg gtataccagt tgtctcacca ggggcaccgc tgga 54

<210> 237
<211> 54
<212> DNA
<213> Mycobacterium smegmatis

<400> 237
ccgggacgga cgaacctctg gtataccagt tgtcccacca ggggcacggc tgga 54

<210> 238
<211> 54
<212> DNA
<213> Mycobacterium tuberculosis

<400> 238
ccgggacgga cgaacctctg gtgcaccagt tgtcccgcca ggggcaccgc tgga 54

<210> 239
<211> 54
<212> DNA
<213> Mycobacterium gallisepticum

<400> 239
ccggagtgaa gacacctctt gtgctccagt tgtagcgcca actgcaccgc tggg 54

<210> 240
<211> 58
<212> DNA
<213> Propionibacterium freudenreichii

<400> 240
ccgggacgga ccaacctctg gtgtgccagt tgttccacca ggagcatggc tggttggc 58

<210> 241
<211> 54
<212> DNA
<213> Rhodococcus erythropolis

<400> 241
ccgggacgga cgaacctctg gtgtgccagt tgttccgcca ggagcaccgc tggt 54

<210> 242
<211> 57
<212> DNA
<213> Rhodococcus fascians

<400> 242
ccgggacgac gaacctctgg tgtgccagtt gttccaccag gagcaccgct ggttggc 57

<210> 243
<211> 58
<212> DNA
<213> Staphylococcus aureus

<400> 243
ccgggatgga catacctctg gtgtaccagt tgtcgtgcca acggcatagc tgggtagc 58

<210> 244
<211> 54
<212> DNA

<213> Streptococcus faecalis

<400> 244
ccgggatgga cttnccgctg gtgtaccagt tgttctgcca agggcattgc tggg 54

<210> 245
<211> 54
<212> DNA
<213> Streptomyces ambifaciens

<400> 245
ccgggatgga cttnccgctg gtgtaccagt tgttctgcca agggcattgc tggg 54

<210> 246
<211> 54
<212> DNA
<213> Flavobacterium resinovorum

<400> 246
ccggagtgga cgtaccgctg gtgtacctgt tgtctcgcca gaggcatcgc aggg 54

<210> 247
<211> 54
<212> DNA
<213> Sphingobacterium multivorans

<400> 247
ccgggttgga cagacctctg gtgaacctgt catnccgcca ggtgtacggc aggg 54

<210> 248
<211> 54
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechococcus

<400> 248
ccggaggaac gcaccgctgg tgtaccagtt atcgtgccaa cggtaaacgc tggg 54

<210> 249
<211> 55
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechocystis

<400> 249
ccgggaagta cgcacctctg gtgtacctgt tatcgtgcca acggtaaacg caggg 55

<210> 250
<211> 59
<212> DNA

<213> Borrelia burgdorferi

<400> 250
ccgagatgga cgaacctcta gtgtaccagt tatcctgcca agggtaagtg ctgggtagc 59

<210> 251
<211> 58
<212> DNA
<213> Chlamydia trachomatis

<400> 251
ccggaatgga cgaaccaatg gtgtgtcggt tgttttgcca agggcatagc cgagtagc 58

<210> 252
<211> 42
<212> DNA
<213> Pseudomonas stutzeri

<400> 252
gagataaccg ctgaaagcat ctaagcggga aacttgcctc aa 42

<210> 253
<211> 41
<212> DNA
<213> Thiobacilluc ferrooxidans

<400> 253
gggataaccg ctgaaagcat ctaagcggaa gccatcctaa g 41

<210> 254
<211> 41
<212> DNA
<213> Agrobacterium vitis

<400> 254
tggataaccg ctgaaggcat ctaagcggga aaccaacctg a 41

<210> 255
<211> 41
<212> DNA
<213> Adalia bipunctata

<400> 255
gggataaccg ctgaatgcat ctaagcagga aactcacctc a 41

<210> 256
<211> 41
<212> DNA
<213> Amycolatopsis orientalis

<400> 256
aggataaccg ctgaaagcat ctaagcggga agcctgcttc g 41

<210> 257
<211> 42

<212> DNA
<213> Brucella ovis

<400> 257
gggataaccg ctgaaggcat ntaagcggga aacccacctg aa 42

<210> 258
<211> 41
<212> DNA
<213> Bradyrhizobium japonicum

<400> 258
gggataaccg ctgaaagcat ctaagcggga aacccacctc a 41

<210> 259
<211> 41
<212> DNA
<213> Pseudomonas paucimobilis

<400> 259
gggataagtg ctgaaagcat ctaagcatga agccccctc a 41

<210> 260
<211> 41
<212> DNA
<213> Rhodobacter sphaeroides

<400> 260
aggataaccg ctgaaggcat ctaagcggga agccccttc a 41

<210> 261
<211> 40
<212> DNA
<213> Rickettsia prowazekii

<400> 261
gggataactg ctgaatgcat ctaagcagga aacccacctc 40

<210> 262
<211> 41
<212> DNA
<213> Sphingomonas paucimobilis

<400> 262
gagataaccg ctgaaagcat ctaagcggga aacttgcctt g 41

<210> 263
<211> 41
<212> DNA
<213> Zymomonas mobilis

<400> 263
gggataaccg ctgaaagcat ctaagcggga agcctccctc a 41

<210> 264

<211> 41
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Alcaligenes

<400> 264
gggataaccg ctgaaagcat ctaagcggga agcctacctc a 41

<210> 265
<211> 41
<212> DNA
<213> Pseudomonas cepacia

<400> 265
gggataaccg ctgaaagcat ctaagcggga agctcgcttc a 41

<210> 266
<211> 41
<212> DNA
<213> Ralstonia pickettii

<400> 266
gagataaccg ctgaaagcat ctaagcggaa aacttgcctc a 41

<210> 267
<211> 41
<212> DNA
<213> Campylobacter jejuni

<400> 267
aggataaacg ctgaaagcat ctaagcgtga agccaactct a 41

<210> 268
<211> 42
<212> DNA
<213> Helicobacter pylori

<400> 268
tgtgataact gctgaaagca tctaagcagg aaccaactcc aa 42

<210> 269
<211> 41
<212> DNA
<213> Actinoplanes utahensis

<400> 269
gggataaccg ctgaaagcat ctaagcggga agctcgcttc g 41

<210> 270
<211> 41
<212> DNA
<213> Bacillus halodurans

<400> 270
gggataagtg ctgaaagcat ctaagcatga agccccctc a 41

<210> 271
<211> 40
<212> DNA
<213> Clostridium tyrobutyricum

<400> 271
gggataaacg ctgaaagcat ctaagcgtga agcccacctc 40

<210> 272
<211> 41
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Frankia

<400> 272
gggataaccg ctgaaagcat ctaagcggga agcctgcttc g 41

<210> 273
<211> 41
<212> DNA
<213> Microbispora bispora

<400> 273
gggataaccg ctgaaagcat ctaagcggga agcccgcccc g 41

<210> 274
<211> 41
<212> DNA
<213> Mycobacterium leprae

<400> 274
aagataaccg ctgaaagcat ctaagcggga aaccttctcc a 41

<210> 275
<211> 41
<212> DNA
<213> Mycobacterium smegmatis

<400> 275
aggataaccg ctgaaagcat ctaagcggga aacctcttcc a 41

<210> 276
<211> 41
<212> DNA
<213> Mycobacterium tuberculosis

<400> 276
aggataaccg ctgaaagcat ctaagcggga aaccttctcc a 41

<210> 277
<211> 41
<212> DNA
<213> Mycobacterium gallisepticum

<400> 277
cggataaacg ctgaaagcat ctaagtgtga aaccgacttt a 41

<210> 278
<211> 43
<212> DNA
<213> Propionibacterium freudenreichii

<400> 278
agtgataacc gctgaaagca tctaagtggg aagcacgctt caa 43

<210> 279
<211> 41
<212> DNA
<213> Rhodococcus erythropolis

<400> 279
gggataaccg ctgaaagcat ctaagcggga agcctgttcc a 41

<210> 280
<211> 41
<212> DNA
<213> Staphylococcus aureus

<400> 280
gggataagtg ctgaaagcat ctaagcatga agccccctc a 41

<210> 281
<211> 41
<212> DNA
<213> Streptococcus faecalis

<400> 281
gggataaacg ctgaaagcat ctaagtgtga agcccncctc a 41

<210> 282
<211> 41
<212> DNA
<213> Streptomyces ambifaciens

<400> 282
gggataaccg ctgaaagcat ctaagcggga agcctgcttc g 41

<210> 283
<211> 41
<212> DNA
<213> Flavobacterium resinovorum

<400> 283
gagataaccg ctgaaagcat ctaagcggga aactcgcctg a 41

<210> 284
<211> 41
<212> DNA
<213> Sphingobacterium multivorans

<400> 284
tagataagcg ctgaaagcat ctaagtgcga aactagccac g 41

<210> 285
<211> 43
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Synechococcus

<400> 285
gtggataacc gctgaaagca tctaagtggg aagcccacct caa 43

<210> 286
<211> 43
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Synechocystis

<400> 286
gtggataacc gctgaaagca tctaagtggg aagcccacct caa 43

<210> 287
<211> 41
<212> DNA
<213> Borrelia burgdorferi

<400> 287
aggataaccg ctgaaagcat ctaagtggga agccttcctc a 41

<210> 288
<211> 41
<212> DNA
<213> Chlamydia trachomatis

<400> 288
aggataagca ttgaaagcat ctaaatgcca agcctccctc a 41

<210> 289
<211> 24
<212> DNA
<213> Pseudomonas stutzeri

<400> 289
agatgagatc tcactggagc cttg 24

<210> 290
<211> 19
<212> DNA
<213> Thiobacillus ferrooxidans

<400> 290
atgagatctc ccgggcata 19

<210> 291
<211> 18
<212> DNA
<213> Agrobacterium vitis

<400> 291
aaacgagtat tccctatc 18

<210> 292
<211> 18
<212> DNA
<213> Adalia bipunctata

<400> 292
aaactagact tccccatc 18

<210> 293
<211> 23
<212> DNA
<213> Amycolatopsis orientalis

<400> 293
agatgagggc tcccacctcc ttg 23

<210> 294
<211> 18
<212> DNA
<213> Brucella ovis

<400> 294
aaacgagtat tccctatc 18

<210> 295
<211> 17
<212> DNA
<213> Bradyrhizobium japonicum

<400> 295
aaacgagcat tcccttg 17

<210> 296
<211> 22
<212> DNA
<213> Pseudomonas paucimobilis

<400> 296
agatgagatt tcccattccg ca 22

<210> 297
<211> 22
<212> DNA
<213> Rhodobacter sphaeroides

<400> 297
agatgagatt tcccattccg ca 22

<210> 298
<211> 18
<212> DNA
<213> Rickettsia prowazekii

<400> 298
aaactagact tccccatt 18

<210> 299
<211> 23
<212> DNA
<213> Sphingomonas paucimobilis

<400> 299
agatgagatt tcccggagcc ttg 23

<210> 300
<211> 14
<212> DNA
<213> Zymomonas mobilis

<400> 300
agataagata tctc 14

<210> 301
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Alcaligenes

<400> 301
agataagatt tccctaggac ttta 24

<210> 302
<211> 23
<212> DNA
<213> Pseudomonas cepacia

<400> 302
agatgagatt tccatacacc ttg 23

<210> 303
<211> 24

<212> DNA
<213> Ralstonia pickettii

<400> 303
agatgagatc tcactggaac cttg 24

<210> 304
<211> 24
<212> DNA
<213> Campylobacter jejuni

<400> 304
agatgaatct tctctaagct ctct 24

<210> 305
<211> 13
<212> DNA
<213> Helicobacter pylori

<400> 305
gataaacttt ccc 13

<210> 306
<211> 23
<212> DNA
<213> Actinoplanes utahensis

<400> 306
agatgaggta tcccaccacc ttg 23

<210> 307
<211> 22
<212> DNA
<213> Bacillus halodurans

<400> 307
agatgagatt tcccatggag ta 22

<210> 308
<211> 22
<212> DNA
<213> Clostridium tyrobutyricum

<400> 308
agattagatt tcccacagcg ta 22

<210> 309
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Frankia

<400> 309

agatgaggtc tcccacaggg tag 23

<210> 310
<211> 23
<212> DNA
<213> Microbispora bispora

<400> 310
agatgaggtc tccctccggg tta 23

<210> 311
<211> 22
<212> DNA
<213> Mycobacterium leprae

<400> 311
agatcaggtt tcttacccac tt 22

<210> 312
<211> 22
<212> DNA
<213> Mycobacterium smegmatis

<400> 312
agaccaggct tctcaccctc ta 22

<210> 313
<211> 22
<212> DNA
<213> Mycobacterium tuberculosis

<400> 313
agatcaggtt tctcacccac tt 22

<210> 314
<211> 30
<212> DNA
<213> Mycobacterium gallisepticum

<400> 314
agaataatct tcccttccag caatggagta 30

<210> 315
<211> 21
<212> DNA
<213> Propionibacterium freudenreichii

<400> 315
gatgagggtt cctgcacagt t 21

<210> 316
<211> 22
<212> DNA
<213> Rhodococcus erythropolis

<400> 316
agatgaggtt tctcaccccc tc 22

<210> 317
<211> 20
<212> DNA
<213> Staphylococcus aureus

<400> 317
agatgagatt tcccaacttc 20

<210> 318
<211> 22
<212> DNA
<213> Streptococcus faecalis

<400> 318
agatgagatt tcccatttct tt 22

<210> 319
<211> 23
<212> DNA
<213> Streptomyces ambifaciens

<400> 319
agatgaggac tcccaccccc ttg 23

<210> 320
<211> 24
<212> DNA
<213> Flavobacterium resinovorum

<400> 320
agatgaggat tccctggcgg cttg 24

<210> 321
<211> 17
<212> DNA
<213> Sphingobacterium multivorans

<400> 321
agatgagact tccttat 17

<210> 322
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechococcus

<400> 322
gatgagtact ctcatggcat 20

<210> 323
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechocystis

<400> 323
gatgagtact ctcatggtgt t 21

<210> 324
<211> 16
<212> DNA
<213> Borrelia burgdorferi

<400> 324
agatgagata tccttt 16

<210> 325
<211> 14
<212> DNA
<213> Chlamydia trachomatis

<400> 325
agataaggta tccc 14

<210> 326
<211> 32
<212> DNA
<213> Pseudomonas stutzeri

<400> 326
agctccctga agggccgtcg aagactacga cg 32

<210> 327
<211> 32
<212> DNA
<213> Thiobacillus ferrooxidans

<400> 327
agcccctga agggacgtgg aagactacca cg 32

<210> 328
<211> 22
<212> DNA
<213> Agrobacterium vitis

<400> 328
agagccgtgg aagacgacca cg 22

<210> 329
<211> 22
<212> DNA
<213> Adalia bipunctata

<400> 329
agagccgtgg aagaccacca cg 22

<210> 330
<211> 30
<212> DNA
<213> Amycolatopsis orientalis

<400> 330
aggggttaag gctcccagta gacgactggg 30

<210> 331
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genera Brucella, Bradyrhizobium

<400> 331
agagccgtgg aagaccacca cg 22

<210> 332
<211> 30
<212> DNA
<213> Pseudomonas paucimobilis

<400> 332
aggaagtaag atccctgaaa gatgatcagg 30

<210> 333
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genera Rhodobacter, Rickettsia

<400> 333
agggccgtgg aagaccacca cg 22

<210> 334
<211> 26
<212> DNA
<213> Sphingomonas paucimobilis

<400> 334
agctccttga agggtcgttc gagacc 26

<210> 335
<211> 22
<212> DNA
<213> Zymomonas mobilis

<400> 335
agagccgtcg aagactacga cg 22

<210> 336
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Alcaligenes

<400> 336
tgtcctctaa agagccgttc gagact 26

<210> 337
<211> 25
<212> DNA
<213> Pseudomonas cepacia

<400> 337
tgtgtgagag gcccccagcc agacc 25

<210> 338
<211> 26
<212> DNA
<213> Ralstonia pickettii

<400> 338
agttccctga agggccgtcg aagact 26

<210> 339
<211> 14
<212> DNA
<213> Campylobacter jejuni

<400> 339
agaagactac tagt 14

<210> 340
<211> 25
<212> DNA
<213> Helicobacter pylori

<400> 340
tgaagctcgc acaaagacta tgtgc 25

<210> 341
<211> 28
<212> DNA
<213> Actinoplanes utahensis

<400> 341
agtgggtaag gctcccagct agactact 28

<210> 342
<211> 31
<212> DNA
<213> Bacillus halodurans

<400> 342
aatccagtaa gaccccttag agatgatgag g 31

<210> 343
<211> 30
<212> DNA
<213> Bacillus subtilis

<400> 343
aggaagtaag atccctgaaa gatgatcagg 30

<210> 344
<211> 32
<212> DNA
<213> Clostridium tyrobutyricum

<400> 344
agctggtaag gcccctttgaa gaacacaagg tg 32

<210> 345
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Frankia

<400> 345
cctggtaagg cccccgacta gatgatcggg 30

<210> 346
<211> 30
<212> DNA
<213> Microbispora bispora

<400> 346
accgggtaag gctcccagta gatgactggg 30

<210> 347
<211> 31
<212> DNA
<213> Mycobacterium leprae

<400> 347
ggtgggataa ggcccccccgc agaacacggg a 31

<210> 348
<211> 31
<212> DNA

<213> Mycobacterium smegmatis

<400> 348
ggagggataa ggccccccgc agaccacggg a 31

<210> 349
<211> 31
<212> DNA
<213> Mycobacterium tuberculosis

<400> 349
ggtgggataa ggccccccgc agaacacggg t 31

<210> 350
<211> 30
<212> DNA
<213> Propionibacterium freudenreichii

<400> 350
aatgtggtaa ggcccccggt agaccaccgg 30

<210> 351
<211> 31
<212> DNA
<213> Rhodococcus erythropolis

<400> 351
gaggggtaa ggcccccggc agaccaccgg g 31

<210> 352
<211> 29
<212> DNA
<213> Staphylococcus aureus

<400> 352
ggttataaga tccctcaaag atgatgagg 29

<210> 353
<211> 31
<212> DNA
<213> Streptococcus faecalis

<400> 353
aagaaagtaa gacccctnan agatgatcag g 31

<210> 354
<211> 30
<212> DNA
<213> Streptomyces ambifaciens

<400> 354
aggggttaag gctcccagta gacgactggg 30

<210> 355
<211> 32

<212> DNA
<213> Flavobacterium resinovorum

<400> 355
accgccttga agggtcgttc gagaccagga cg 32

<210> 356
<211> 22
<212> DNA
<213> Sphingobacterium multivorans

<400> 356
agggtcgtag aagatgacta cg 22

<210> 357
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Synechococcus

<400> 357
aagccagtaa ggtcacgggt agaacacccg 30

<210> 358
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Synechocystis

<400> 358
aagccagtaa ggtcacggga agactacccg 30

<210> 359
<211> 23
<212> DNA
<213> Borrelia burgdorferi

<400> 359
aagggtcctg gaagaatacc agg 23

<210> 360
<211> 26
<212> DNA
<213> Chlamydia trachomatis

<400> 360
aatgagactc catgtagact acgtgg 26

<210> 361
<211> 40

<212> DNA
<213> Pseudomonas stutzeri

<400> 361
agtaatgcat taagctaacc agtactaatt gcccgtacgg 40

<210> 362
<211> 40
<212> DNA
<213> Thiobacillus ferrooxidans

<400> 362
agcaatgcgt gcagctaagg agtactaatc ggccgtgcgg 40

<210> 363
<211> 40
<212> DNA
<213> Agrobacterium vitis

<400> 363
ggtaacctgc gaagcttacc gttactaata gctcgattgg 40

<210> 364
<211> 40
<212> DNA
<213> Adalia bipunctata

<400> 364
agtaatgcgt gtagctaacc gatactaata gctcgattga 40

<210> 365
<211> 40
<212> DNA
<213> Brucella ovis

<400> 365
ggcaacgcat gcagcttacc ggtactaata gctcgatcga 40

<210> 366
<211> 40
<212> DNA
<213> Bradyrhizobium japonicum

<400> 366
agtaatgcat gcagcttacc ggtactaatc gttcgattgg 40

<210> 367
<211> 40
<212> DNA
<213> Pseudomonas paucimobilis

<400> 367
ggcgacacat ggagctgaca gatactaatc gatcgaggac 40

<210> 368

<211> 40
<212> DNA
<213> Rhodobacter sphaeroides

<400> 368
agcaatgcgt tcagctgact ggtactaatt gcccgatagg 40

<210> 369
<211> 40
<212> DNA
<213> Rickettsia prowazekii

<400> 369
agtaatgtgt gtagctaacc gatactaata gctcgattga 40

<210> 370
<211> 40
<212> DNA
<213> Sphingomonas paucimobilis

<400> 370
agtaatgcat taagctaacc agtactaatt gcccgtncgg 40

<210> 371
<211> 40
<212> DNA
<213> Zymomonas mobilis

<400> 371
ggtaacacat gtagctaact ggtcctaatt gctctattca 40

<210> 372
<211> 40
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived from species of the genus Alcaligenes

<400> 372
agtgatatgt gaagctgacc aatactaatt gctcgtgagg 40

<210> 373
<211> 40
<212> DNA
<213> Ralstonia pickettii

<400> 373
tgtgaggcgt tgagctaacc aatactaatt gcccgtgagg 40

<210> 374
<211> 40
<212> DNA
<213> Campylobacter jejuni

<400> 374
tgaaagtcct ttagctgacc agtactaata gagcgtttgg 40

<210> 375
<211> 40
<212> DNA
<213> Helicobacter pylori

<400> 375
agtaatgcgt ttagctgact actactaata gagcgtttgg 40

<210> 376
<211> 40
<212> DNA
<213> Bacillus halodurans

<400> 376
ggcgacacgt gaagctgaca gatactaatc ggtcgaggac 40

<210> 377
<211> 40
<212> DNA
<213> Bacillus subtilis

<400> 377
ggcgacacat ggagctgaca gatactaatc gatcgaggac 40

<210> 378
<211> 40
<212> DNA
<213> Clostridium tyrobutyricum

<400> 378
ggcaacatgt tcagctgact gatactaata ggccgagggc 40

<210> 379
<211> 41
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Frankia

<400> 379
cggtgacgca tggagctgac cggtactaat aggccgaggg c 41

<210> 380
<211> 42
<212> DNA
<213> Microbispora bispora

<400> 380
cggtaacgtg tggagccgac cggtactaat aagccgagag gc 42

<210> 381
<211> 41
<212> DNA
<213> Mycobacterium leprae

<400> 381
cagtaatgag tgtagggaac tggcactaac tggccgaaag c 41

<210> 382
<211> 41
<212> DNA
<213> Mycobacterium smegmatis

<400> 382
tagtaatagg tgcagggaac tggcactaac cggccgaaaa c 41

<210> 383
<211> 41
<212> DNA
<213> Mycobacterium tuberculosis

<400> 383
cagtaatggg tgtagggaac tggtgctaac cggccgaaaa c 41

<210> 384
<211> 86
<212> DNA
<213> Mycobacterium gallisepticum

<400> 384
agaatcgttg tagactacga cgttgatagg ctaaaggtgt aagtgccgcg aggtatttag 60
ctgattagta ctaataattc gaggac 86

<210> 385
<211> 27
<212> DNA
<213> Propionibacterium freudenreichii

<400> 385
gctgaccgat actaagtggc cgagggc 27

<210> 386
<211> 41
<212> DNA
<213> Rhodococcus erythropolis

<400> 386
cagtaatgca tgcaggtgac tggtactaat aggccgagga c 41

<210> 387
<211> 41
<212> DNA
<213> Rhodococcus fascians

<400> 387
cagcaatgta tgcaggtgac tggtactaat aggccgagga c 41

<210> 388
<211> 27
<212> DNA
<213> Staphylococcus aureus

<400> 388
gctgacgaat actaatcgat cgagggc 27

<210> 389
<211> 27
<212> DNA
<213> Streptococcus faecalis

<400> 389
gcggaccaat actaatcggt cgaggac 27

<210> 390
<211> 51
<212> DNA
<213> Streptomyces ambifaciens

<400> 390
ccgcaaggtg tggaggtgac cggtactaat aggccgaggg cttgtcctca t 51

<210> 391
<211> 51
<212> DNA
<213> Streptomyces galbus

<400> 391
cggtaacgtg tggaggtgac cggtactaat aggccgaggg cttgtcctca g 51

<210> 392
<211> 51
<212> DNA
<213> Streptomyces griseus

<400> 392
cggtaacggg tggagctgac tggtactaat aggccgaggg cttgtcctca g 51

<210> 393
<211> 51
<212> DNA
<213> Streptomyces lividans

<400> 393
ccgtgaggtg tggaggtgac cggtactaat aggccgaggg cttgtcctca g 51

<210> 394
<211> 51
<212> DNA
<213> Streptomyces mashuensis

<400> 394

cggtaacggt tggagctgac tggtactaat aggccgaggg cttgtccata g 51

<210> 395
<211> 28
<212> DNA
<213> Flavobacterium resinovorum

<400> 395
gctaaccagt actaattgcc cgtaaggc 28

<210> 396
<211> 28
<212> DNA
<213> Sphingobacterium multivorans

<400> 396
gccaagtggt actaatagcc cgaagctt 28

<210> 397
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechococcus

<400> 397
gctgaggcgt actaatagac cgagggc 27

<210> 398
<211> 27
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechocystis

<400> 398
gtcgaggagt actaatagac cgagggc 27

<210> 399
<211> 27
<212> DNA
<213> Borrelia burgdorferi

<400> 399
gctgactaat actaattacc cgtatct 27

<210> 400
<211> 28
<212> DNA
<213> Chlamyia trachomatis

<400> 400

gctaaccaat actaataagt ccaaagac 28

<210> 401
<211> 36
<212> DNA
<213> Salmonella typhi

<400> 401
cttaacctta caacgccgaa gatgttttgg cggatg 36

<210> 402
<211> 35
<212> DNA
<213> Buchnera aphidocola

<400> 402
cttaacctta caacaccaga ggtgtttttt ataaa 35

<210> 403
<211> 35
<212> DNA
<213> Pseudomonas stutzeri

<400> 403
cttgaccata taacacccaa acaatttgat gtttg 35

<210> 404
<211> 35
<212> DNA
<213> Thiobacillus ferrooxidans

<400> 404
cttgaccata tatcaccaag cattaaagag cttcc 35

<210> 405
<211> 35
<212> DNA
<213> Sphingomonas paucimobilis

<400> 405
cttgtcccta taaccttggt agtccaaggt cgagt 35

<210> 406
<211> 35
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Alcaligenes

<400> 406
cttgactata caacacccaa gcagttgtat ataaa 35

<210> 407

<211> 23
<212> DNA
<213> Pseudomonas cepacia

<400> 407
aggactaacg actcgtgaag ctg 23

<210> 408
<211> 29
<212> DNA
<213> Ralstonia pickettii

<400> 408
cttgaccata taacacccaa gcaatttga 29

<210> 409
<211> 35
<212> DNA
<213> Campylobacter jejuni

<400> 409
cttatcttta ataaagcatc acttccttgt taagg 35

<210> 410
<211> 35
<212> DNA
<213> Helicobacter pylori

<400> 410
cttgtttttt gctttttgat aagataacgg caata 35

<210> 411
<211> 33
<212> DNA
<213> Actinoplanes utahensis

<400> 411
cggtaacgtg ttgagttgac cggtactaat agg 33

<210> 412
<211> 35
<212> DNA
<213> Bacillus halodurans

<400> 412
ttatccaaaa acaaatcaaa agcaacgtct cgaac 35

<210> 413
<211> 21
<212> DNA
<213> Bacillus subtilis

<400> 413
ttaaccacat tttgaatgat g 21

<210> 414
<211> 32
<212> DNA
<213> Clostridium tyrobutyricum

<400> 414
ttgaccaaat ttatcttact gtgcaatttt ca 32

<210> 415
<211> 56
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Frankia

<400> 415
cggtgacgca tggagctgac cggtactaat aggccgaggg cttgtcttcg aaggtg 56

<210> 416
<211> 56
<212> DNA
<213> Microbispora bispora

<400> 416
cggtaacgtg tggagccgac cggtactaat aagccgagag gcttgacttc acatgc 56

<210> 417
<211> 56
<212> DNA
<213> Mycobacterium leprae

<400> 417
cagtaatgag tgtagggaac tggcactaac tggccgaaag cttacaaaac acacac 56

<210> 418
<211> 56
<212> DNA
<213> Mycobacterium smegmatis

<400> 418
tagtaatagg tgcagggaac tggcactaac cggccgaaaa cttacaacac cccata 56

<210> 419
<211> 56
<212> DNA
<213> Mycobacterium tuberculosis

<400> 419
cagtaatggg tgtagggaac tggtgctaac cggccgaaaa cttacaacac cctccc 56

<210> 420
<211> 39
<212> DNA
<213> Mycobacterium gallisepticum

<400> 420
cgttgatagg ctaaaggtgt aagtgccgcg aggtattta 39

<210> 421
<211> 39
<212> DNA
<213> Propionibacterium freudenreichii

<400> 421
ttgtcccaca ctttaattct tgtagattgt tgtgaagag 39

<210> 422
<211> 41
<212> DNA
<213> Rhodococcus erythropolis

<400> 422
cagtaatgca tgcaggtgac tggtactaat aggccgagga c 41

<210> 423
<211> 41
<212> DNA
<213> Rhodococcus fascians

<400> 423
cagcaatgta tgcaggtgac tggtactaat aggccgagga c 41

<210> 424
<211> 33
<212> DNA
<213> Staphylococcus aureus

<400> 424
ttaaccaaaa taaatgtttt gcgaagcaaa atc 33

<210> 425
<211> 42
<212> DNA
<213> Streptococcus faecalis

<400> 425
ttaaccaaag aatggataag taaaagcaac ttggttattt tg 42

<210> 426
<211> 56
<212> DNA
<213> Streptomyces lividans

<400> 426
ccgcaaggtg tggaggtgac cggtactaat aggccgaggg cttgtcctca tttgct 56

<210> 427
<211> 56
<212> DNA

<213> Streptomyces mashuensis

<400> 427
cggtaacggt tggagctgac tggtactaat aggccgaggg cttgtccata gttgct 56

<210> 428
<211> 43
<212> DNA
<213> Flavobacterium resinovorum

<400> 428
cttgatccta taaccagtgt gttttgcctg gtgggtgatc gcg 43

<210> 429
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechococcus

<400> 429
ttgacctcta acactttgat atcggcac 28

<210> 430
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechocystis

<400> 430
ttgaccttta ttcttcattt ttcttttct 28

<210> 431
<211> 34
<212> DNA
<213> Chlamyia trachomatis

<400> 431
cttggtcttt ttatgattgg aagagccgaa aggc 34

<210> 432
<211> 51
<212> DNA
<213> Salmonella typhi

<400> 432
cttaaccttа caacaccgaa ggtgttttgg aggataaaag aaacagaatt t 51

<210> 433
<211> 117
<212> DNA

<213> Buchnera aphidocola

<400> 433
cttaacctta caacaccaga ggtgtttttt ataaaaaata aaaaatcttg ttttactgaa 60
tttattgttg tattaatata tatatattat aatagcacta aaaaatgcct ggtaaaa 117

<210> 434
<211> 233
<212> DNA
<213> Pseudomonas stutzeri

<400> 434
cttgaccata taacacccaa acaatttgat gtttgcgtgt cagacggttg aagtcgacaa 60
acaaaccgaa agacgcaacg ctcgcaaagc gaaagcgata ccgaagcaac catcacatac 120
ccaattaggg aagcgactca acaccgactc cccagttgaa cttgcttgac gaccatagag 180
cgttggaacc acctgatccc atcccgaact cagtagtgaa acgacgcatc gcc 233

<210> 435
<211> 91
<212> DNA
<213> Thiobacillus ferrooxidans

<400> 435
cttgaccata tatcaccaag cattaaagag cttcccttca gcaacacctc gagggcggca 60
cagccgcgcc cgggaccaga ccagttttaa c 91

<210> 436
<211> 230
<212> DNA
<213> Agrobacterium vitis

<400> 436
cttaatcgtt ctcattgacc atgctcatcg acttcgtcga tgagccatct gtttagcgct 60
cacgcatgag cggctcgtat acgagcctat gctccgcgag ggcgccgaac gatcggcgac 120
gcgccttgcg cttgcggact tcgtccgaaa gtgccaagca aaacgtcgcg gaatgacgtg 180
ttcacacaat aagaaaacgg gcaatgcccg ccagcttctc atcaacattg 230

<210> 437
<211> 162
<212> DNA
<213> Adalia bipunctata

<400> 437
tttactttgc tgtgagatta cacatgcata tggtgttaat tctataaaca tgtaagtatc 60
aactcacaaa gttatcaggt taaattagct ttatcaacca ataaagatgt tgttacatgt 120
ctctttctat gttgttcctg tgaaagtaag aatctagaaa aa 162

<210> 438
<211> 120
<212> DNA
<213> Amycolatopsis orientalis

<400> 438
tggtaacggg tggagttgac tggtactaat aggccgaggg cttgtcctca gttgctcgcg 60
tccactgtgt tagttctgaa gtaacgaaca tcgccttgtc ggctggagtt caacttcata 120

<210> 439
<211> 189
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Brucella

<400> 439
cttgatcact cccatttaca atatccatca agcaaaagct tgatgttgaa ggcaatatgg 60
aagtagggca ataaggcaat atgtttgccc aaagccctca accatcgcca cgcagaaaaa 120
caaagcacaa aggcaaagaa caggcgcagc ccaaacatac tgccctattc ccctaatgcc 180
ttaagcccc 189

<210> 440
<211> 109
<212> DNA
<213> Bradyrhizobium japonicum

<400> 440
cttgattgct ctcattttca gtgtccatag ggccgcaagg cccgcgacca gaatgaaatg 60
agaggcgcta gtcgcccaac aaagatcgct tgcttcgtat tccttgtcc 109

<210> 441
<211> 125
<212> DNA
<213> Pseudomonas paucimobilis

<400> 441
cttaaccaat ttgaatgtat gcttactgtt atctagtttt gagagaacac tctcaatggt 60
ttggtggcga tagcgaagag gtcacacccg ttcccatgcc gaacacggaa gttaagctct 120
tcagc 125

<210> 442
<211> 100
<212> DNA
<213> Rhodobacter sphaeroides

<400> 442
cttgatctga cccggtaaca gcaaggctca aaagccaacg ctctacccca gatcagaagc 60
aatagacccg gaacaagcaa aagcctgatg ttgtcgtttc 100

<210> 443
<211> 196
<212> DNA
<213> Rickettsia prowazekii

<400> 443
tttactttgc tgtgagatta tatatgcata tagtgttaat tatataagta tttaagcatc 60
aatttgtaaa ttataatttt aatgttaaat tagctttatc aataaataaa aatgttattc 120
tatcgtttta tgttacgatt tgatagtaaa gttttgatct ttctttaaga tattgtagac 180
aattgtatat tatacc 196

<210> 444
<211> 249
<212> DNA

<213> Pseudomonas cepacia

<400> 444
aggactaacg actcgtgaag ctgaccggta ctaataggcc gataacttac accacacacc 60
cttttcgtga acggattcaa aagacgttca caccaggaga gggtaaaaag aaaaaacaag 120
actgcttgcg tccactatgt ggttcccaac caacaaaccc gccacgggca cgttgcgaca 180
ggaacacaac tgaataacaa caccacaatg ttgtaaccac aaagacttcc caccccggc 240
atcagaccc 249

<210> 445
<211> 209
<212> DNA
<213> Ralstonia pickettii

<400> 445
cttgaccata taacacccaa gcaatttgag cgtaggcgcc aaattgtggt ggtgaagatg 60
atacgaaccg aaagttcgca acgaaccaca acatcacata tccgaattcg ctgggctgtc 120
catctggaca ttctggctac agaatttctt gacgaccata gagcattgga accacctgat 180
cccatcccga actcagcagt gnaacgatg 209

<210> 446
<211> 271
<212> DNA
<213> Campylobacter jejuni

<400> 446
cttatcttta ataaagcatc acttccttgt taaggttttt aagaagactt tgaatataga 60
taatatttag agtttaatag aaatctttca agtaaagttt gtattagaac ttgctcttaa 120
cattgttttt taagtattct atataaaaac ttatcaaaga taaaagataa gaaaagaaga 180
aagagaataa aagattaagt tttattctta aattcaattt ttcaaagaat atttaaataa 240
caatgtccgt gattatacag atgtggaaac g 271

<210> 447
<211> 228
<212> DNA
<213> Helicobacter pylori

<400> 447
cttgtttttt gctttttgat aagataacgg caataagcgc gaatgggtta ccactgcctt 60
actgagtgta agagagttgg agttttatga agacttttat aagattaaac tttaatgagg 120
aatgagatac catctcaatg gtttaaagtt aaaggctatt aacgatcttc tttgttaaaa 180
acagctcccc tataaagaga aaggggagtt aagggtaaat gcgttttt 228

<210> 448
<211> 155
<212> DNA
<213> Actinoplanes utahensis

<400> 448
cggtaacgtg ttgagttgac cggtactaat aggccgaggg cttaaccacc ctaaattttc 60
tgcttgcgtc cactgtgtga ttcacagcaa acgaacaacc accccggttc aagagtgccg 120
ggttgctggt ttgttctgct gatggctgtt tcgat 155

<210> 449
<211> 296
<212> DNA
<213> Bacillus halodurans

<400> 449
cttatccaaa aacaaatcaa aagcaacgtc tcgaactcga gaagcgtccc attatctagt 60
tttgagagaa tcttgttctc caaagaagcg ctccgacgca gcatcgcaag atgcgaagtt 120
gatcggaagc cgtgatcaag agattattct cttaggtcca aagaaaaggg tttcgagaaa 180
cgagcagttt taggaatcga gcgacgacag atcggagcgt acacacggta cgtgaggatc 240
tggaggagtg aagatgacac caaaatgcga tgttgatcgg aggccgtaac tatcta 296

<210> 450
<211> 122
<212> DNA
<213> Bacillus halodurans

<400> 450
cttaaccaca ttttgaatga tgtcacacct gttatctagt tttgagagaa cacctctcta 60
aaggcggaag gtaaggaaac tccgctaagg gctctcacat cctgtgagaa acgcccagta 120
cc 122

<210> 451
<211> 209
<212> DNA
<213> Clostridium tyrobutyricum

<400> 451
cttgaccaaa tttatcttac tgtgcaattt tcagagaata attattctct tatctccatt 60
agaaatataa tgtttctatt ttattataga gaataaagta agtaaattga taataaccat 120
tagtacaagg aagatatgag cgaagagcgg aatttactta ggtaaatgag cactggagtg 180
aataattctg acggtgtaat gagaagtta 209

<210> 452
<211> 100
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Frankia

<400> 452
cggtgacgca tggagctgac cggtactaat aggccgaggg cttgtcttcg aaggtgctac 60
gcgtccactg tgcggttctc gggtgtacgg ccggttcggc 100

<210> 453
<211> 85
<212> DNA
<213> Microbispora bispora

<400> 453
cggtaacgtg tggagccgac cggtactaat aagccgagag gcttgacttc acatgcacgc 60
acccactatg cgattctcga tcagc 85

<210> 454
<211> 124
<212> DNA
<213> Mycobacterium leprae

<400> 454

cagtaatgag tgtagggaac tggcactaac tggccgaaag cttacaaaac acacacatcg 60
caaccacata attcagatcc actttgtcgt ggagcatcac acccccatc agaacaaatt 120
ttta 124

<210> 455
<211> 146
<212> DNA
<213> Mycobacterium smegmatis

<400> 455
tagtaatagg tgcagggaac tggcactaac cggccgaaaa cttacaacac cccataatcg 60
ttgtaagaag aaaacattga cgcaccgcgc tcgcaaccac actccacgga tgatcaaacc 120
cacaagtttg ctctccatgt gggtca 146

<210> 456
<211> 135
<212> DNA
<213> Mycobacterium tuberculosis

<400> 456
cagtaatggg tgtagggaac tggtgctaac cggccgaaaa cttacaacac cctccctttt 60
ggaaaaggga ggcaaaaaca aactcgcaac cacatccgtt cacggcgcta gccgtgcgtc 120
cacacccccc accag 135

<210> 457
<211> 169
<212> DNA
<213> Mycobacterium gallisepticum

<400> 457
cgttgatagg ctaaaggtgt aagtgccgcg aggtatttag ctgattagta ctaataattc 60
gaggacttag atttgatcaa aaacattagc tgtttttttat ctaatatgat ttgttgtatt 120
ttgtttttca aagagcaatg tgtgtgatat cgatatcgtg atggaaaca 169

<210> 458
<211> 43
<212> DNA
<213> Propionibacterium freudenreichii

<400> 458
cttgtcccac actttaattc ttgtagattg ttgtgaagag ttt 43

<210> 459
<211> 182
<212> DNA
<213> Rhodococcus erythropolis

<400> 459
cagtaatgca tgcaggtgac tggtactaat aggccgagga cttaccacaa agaagctacg 60
cgtccactgt gcggtatctg aaacaacaca cagatactga tgagaaaccc tgttttctcc 120
atcccccaac accagaaact ggtgttgacg tggtgaaacc aggtgatcag aagaaggtta 180
ct 182

<210> 460
<211> 168
<212> DNA

<213> Rhodococcus fascians

<400> 460
cagcaatgta tgcaggtgac tggtactaat aggccgagga cttaccacaa agaagctacg 60
cgtccactgt gcaatatctg aaacaacaca cgagtagttg ttcgacaaca gaaccgaata 120
cacgaatccg ccacccacac gagtgtgggt gacaggttcg ctcgttga 168

<210> 461
<211> 64
<212> DNA
<213> Staphylococcus aureus

<400> 461
cttaaccaaa ataaatgttt tgcgaagcaa aatcactttt acttactatc tagttttgaa 60
tgta 64

<210> 462
<211> 87
<212> DNA
<213> Streptococcus faecalis

<400> 462
cttaaccaaa gaatggataa gtaaaagcaa cttggttatt ttgattcaaa cttcaatcca 60
gttttgagtg aatnaagatt cnctcaa 87

<210> 463
<211> 123
<212> DNA
<213> Streptomyces ambifaciens

<400> 463
ccgcaaggtg tggaggtgac cggtactaat aggccgaggg cttgtcctca tttgctcgcg 60
tccactgtgt tggttctgaa accacgaaca accccatgtg ccacacatgg tgcggttgtc 120
agt 123

<210> 464
<211> 134
<212> DNA
<213> Streptomyces galbus

<400> 464
cggtaacgtg tggaggtgac cggtactaat aggccgaggg cttgtcctca gttgctcgcg 60
tccactgtgt tggttctgaa accacgaaca gccccatgct ctggcatggt gcggcattgt 120
tcgacagttt cata 134

<210> 465
<211> 143
<212> DNA
<213> Streptomyces griseus

<400> 465
cggtaacggg tggagctgac tggtactaat aggccgaggg cttgtcctca gttgctcgcg 60
tccactgtgt tgttcccggg ttgcgaacag ttatcgcacc ggttgaacag tttcactact 120
taattgaaga gtgtgcttgt tcg 143

<210> 466

<211> 137
<212> DNA
<213> Streptomyces lividans

<400> 466
ccgtgaggtg tggaggtgac cggtactaat aggccgaggg cttgtcctca gttgctcgcg 60
tccactgtgt tagttctgag gcaacgaccg ttgccggatt tgagtagaac gcacaattaa 120
agagtgtgct tgttcgc 137

<210> 467
<211> 135
<212> DNA
<213> Streptomyces mashuensis

<400> 467
cggtaacggt tggagctgac tggtactaat aggccgaggg cttgtccata gttgctcgcg 60
ttcactgtgt tggttctgaa acaacaacca agaagcatac gccgtgtgtg gttgacagtt 120
tcatagtgtt tcggt 135

<210> 468
<211> 114
<212> DNA
<213> Flavobacterium resinovorum

<400> 468
cttgatccta taaccagtgt gttttgcctg gtgggtgatc gcgactgtgc cgaaacagtt 60
gacacgcaca accccaacta catccctatt cgcagcgttg acctcaacct cagc 114

<210> 469
<211> 126
<212> DNA
<213> Sphingobacterium multivorans

<400> 469
ctttctcaag cagataacac tgttgtcttc ctctttaatt tttagaaacg aaaagaataa 60
caaaaaagaa acgaagctct ttcaatagat atgtcagttg gcctgacgat gatatattat 120
cataag 126

<210> 470
<211> 63
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechococcus

<400> 470
cttgacctct aacactttga tatcggcact ctcctctatg cagccttcaa ggctctaatc 60
tcc 63

<210> 471
<211> 67
<212> DNA
<213> Artificial sequence

<220>

<223> Description of the artificial sequence: derived
from species of the genus Synechocystis

<400> 471
cttgaccttt attcttcatt tttctttctc ttttcttgtg cagtcttctg ggtttcttct 60
cagcaaa 67

<210> 472
<211> 17
<212> DNA
<213> Borrelia burgdorferi

<400> 472
ctttggccat atttttg 17

<210> 473
<211> 111
<212> DNA
<213> Chlamydia trachomatis

<400> 473
cttggtcttt ttatgattgg aagagccgaa aggcaaagac aataagaaaa agagtagaga 60
gtgcaagtac gtagaagaca agcttttaag cgtctattag tatacgtgag a 111

<210> 474
<211> 148
<212> DNA
<213> Azotobacter vinelandii

<400> 474
aaacaatctg ttgccagccc cagcggggcg gcacggagag ggcgcagccg acaggccgaa 60
gatttggctg gaccgcacgc tgccggaaac aggctaccgc tatcacctac ccgattggct 120
gtcgtgtcat cgacacggcg gcaaccga 148

<210> 475
<211> 229
<212> DNA
<213> Cowduria ruminantium

<400> 475
ggtgtgtaag tatggtaaca tatgtagcta accagtacta atagcccgat tgatttactt 60
aatttgtaat tatatgtagt attaaaactg cagcttgtct ttttgcttat tttgttttat 120
agtttaattg ggttggtggt aatagcagaa gtgatacacc cagctacatt tcgaacctgg 180
aagttaagcc ttctagcgct tatggtactt tgtcttaagg cacgggaga 229

<210> 476
<211> 110
<212> DNA
<213> Mycobacterium intracellulare

<400> 476
taagcttgat tcacacactc gcaaccacag tccatttcgc gcgttctgcc gctgaagcta 60
gaacaccgca ccccccacca aacaaattta aatagagtta cggcggccac 110

<210> 477
<211> 107

<212> DNA
<213> Mycobacterium lufu

<400> 477
aaaacttacc gaacacacaa tcgcaaccac agtccatttc acggcagcaa tgccgcgaaa 60
cgccacaccc cccaccaaac aaatttaaat agagttacgg cggccac 107

<210> 478
<211> 120
<212> DNA
<213> Mycobacterium simiae

<400> 478
taagcttgat tcacacacat cgcaaccact atcgtcgcga cttattgtcg cgccgaatgc 60
cacacccccc accagaacaa ctaataaaat agtgttccgt aatagagtta cggcggccac 120

<210> 479
<211> 149
<212> DNA
<213> Mycobacterium smegmatis

<400> 479
caccccataa cgttgtaaga agaaaacatt gaccaccgcg ctcgcaacca cactccacgg 60
atgatcaaac cgatcacccc accaccaaaa caaacccaca agtttgctct ccatgtgggt 120
caccacataa gagaatagag ttacggcgg 149

<210> 480
<211> 75
<212> DNA
<213> Saccharomonospora azurea

<400> 480
caaagatgct acgcacccac tctgcaactc tgaaacacca caccccggaa acatgatcct 60
gggttgtttc acagt 75

<210> 481
<211> 73
<212> DNA
<213> Saccharomonospora caesia

<400> 481
caaagatgct acgcacccac tctgcaactc tgaaacacca caccccggaa acgatcctgg 60
gttgtttcac agt 73

<210> 482
<211> 75
<212> DNA
<213> Saccharomonospora cyanea

<400> 482
caaacatgct acgcacccac tctgcaactc tgaaacacca ccccgggaac acacccggcg 60
tgattgtttc ccaga 75

<210> 483
<211> 69
<212> DNA

<213> Saccharomonospora glauca

<400> 483
caaagacgct acgcacccac tctgcgactc tgaaacacca ccctggtgtg ccagtggttg 60
tttcacaga 69

<210> 484
<211> 74
<212> DNA
<213> Saccharomonospora viridis

<400> 484
caaaggtgct acgcacccac tctgcaactc tgaaacacca caccccacaa acaccgggct 60
ggttgtttca caga 74

<210> 485
<211> 304
<212> DNA
<213> Wolbachia pipientis

<400> 485
taactggtac taatagcctg attgatttat ttgctttcta tatgtgcata tgcagtgtta 60
aatattaagt taaaatttat taagtcagaa atttttgttg acttggtggc tatagcaaaa 120
atgaaccacc cgatctcatt tcgaactcgg aagtgaaact ttttagcgcc gatgatactt 180
aaaaacccaa agtaggtcgt tgccaagttt ataaaaattt cttcttattt atatcttttc 240
agtagagcga tgaaacaagg taaacataga gtagctgtga ggtaatataa ctgatctttt 300
agaa 304

<210> 486
<211> 34
<212> DNA
<213> Salmonella typhi

<400> 486
ttcctggcgg cactagcgcg gtggtcccac ctga 34

<210> 487
<211> 22
<212> DNA
<213> Buchnera aphidocola

<400> 487
atagtgtagt ggtaccacct ga 22

<210> 488
<211> 53
<212> DNA
<213> Pseudomonas stutzeri

<400> 488
catcgccgat ggtagctgtg gggtctcccc atgtgagagt aggtcatcgt caa 53

<210> 489
<211> 35
<212> DNA
<213> Thiobacillus ferrooxidans

<400> 489
cttgtctggc ggccatagcg cagtggaacc acccc 35

<210> 490
<211> 52
<212> DNA
<213> Agrobacterium vitis

<400> 490
atcaacattg cccttagctg acctggtggt catggcgggg cggccgcacc cg 52

<210> 491
<211> 38
<212> DNA
<213> Adalia bipunctata

<400> 491
gccatgcaac aatgttaaca gcagactaat acaaatct 38

<210> 492
<211> 52
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Brucella

<400> 492
atgtttgtgt tcttcgccga cctggtggtt atggcggagc ggccgcaccc ga 52

<210> 493
<211> 40
<212> DNA
<213> Bradyrhizobium japonicum

<400> 493
ttcgccggcc tggtggtttt agcgaagagc ctcaacccga 40

<210> 494
<211> 36
<212> DNA
<213> Pseudomonas paucimobilis

<400> 494
tcttcagcgc cgatggtagt cggggttccc cctaat 36

<210> 495
<211> 40
<212> DNA
<213> Rhodobacter sphaeroides

<400> 495
ttctccggtc tggtggccat agcacgagca aaacacccga 40

<210> 496
<211> 53
<212> DNA
<213> Rickettsia prowazekii

<400> 496
ccttgcttaa gaataatata atagcattaa cagcatatta taatacaacc tat 53

<210> 497
<211> 51
<212> DNA
<213> Rickettsia bellii

<400> 497
aaatttcttt aagtcctgca acaacactaa cagcaaacca atacaaatct a 51

<210> 498
<211> 53
<212> DNA
<213> Rickettsia rickettsii

<400> 498
gaatttttt gagtcgtgca acaacattaa cagtagacta taatacaaat cta 53

<210> 499
<211> 47
<212> DNA
<213> Sphingomonas paucimobilis

<400> 499
gccagacaag tcaaagcctg atgaccatag caagtcggtc ccacccc 47

<210> 500
<211> 33
<212> DNA
<213> Zymomonas mobilis

<400> 500
gcttggtggc tatagcgtca gtgacccacc cga 33

<210> 501
<211> 53
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Alcaligenes

<400> 501
gcaagtatcc ataccagttg tgctggcgac catagcaaga gtgaaccacc tga 53

<210> 502
<211> 51
<212> DNA

<213> Pseudomonas cepacia

<400> 502
cgggcggacg ggtacaaggg ttacggcggt catagcgtgg gggaaacgcc c 51

<210> 503
<211> 48
<212> DNA
<213> Ralstonia pickettii

<400> 503
catcgccgat ggtagtgtgg ggtttcccca tgcgagagta ggacatag 48

<210> 504
<211> 51
<212> DNA
<213> Helicobacter pylori

<400> 504
ttatctttag ctccctttc cttgtgcctt tagagaagag gaactaccca g 51

<210> 505
<211> 52
<212> DNA
<213> Bacillus halodurans

<400> 505
caaagaggat caagagattt gcggaagcaa gcgagtgacg aactgagcgt at 52

<210> 506
<211> 52
<212> DNA
<213> Bacillus halodurans

<400> 506
ccttcatcct gaaggcattt gtttggtggc gatagcgaag aggtcacacc cg 52

<210> 507
<211> 52
<212> DNA
<213> Clostridium tyrobutyricum

<400> 507
ttagcagcaa tttacggttg atctggtaac aatgacgtga aggtaacact cc 52

<210> 508
<211> 51
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Frankia

<400> 508
ggttgtatag ttgaatagtg tttcggtggt tttggcgaag gggaaacgcc c 51

<210> 509
<211> 50
<212> DNA
<213> Microbispora bispora

<400> 509
gtcctcacct gaaggcttgc cgctatcccg cgtcgagcag gtgaattccg 50

<210> 510
<211> 45
<212> DNA
<213> Mycobacterium leprae

<400> 510
aattttatag agttacggtg gccacagcga tagggaaacg cccgg 45

<210> 511
<211> 52
<212> DNA
<213> Mycobacterium smegmatis

<400> 511
accacataag agaatagagt tacggcggtc catagcggca gggaaacgcc cg 52

<210> 512
<211> 49
<212> DNA
<213> Mycobacterium tuberculosis

<400> 512
agaacaaatt tgcatagagt tacggcggcc acagcggcag ggaaacgcc 49

<210> 513
<211> 51
<212> DNA
<213> Rhodococcus erythropolis

<400> 513
ctgtgacagt ttcatagagt tacggcggtc atagcgaagg ggaaacgccc g 51

<210> 514
<211> 52
<212> DNA
<213> Rhodococcus fascians

<400> 514
ttgacactgt ttcgcagagt tacggcggcc atagcggagg ggaaaccgcc cg 52

<210> 515
<211> 53
<212> DNA
<213> Staphylococcus aureus

<400> 515

tgtataaatt acattcatat gtctggtgac tatagcaagg aggtcacacc tgt 53

<210> 516
<211> 50
<212> DNA
<213> Streptococcus faecalis

<400> 516
taagaaacaa cacccagtgt ggtggcgata gcgagaagga tacacctgtt 50

<210> 517
<211> 47
<212> DNA
<213> Streptomyces ambifaciens

<400> 517
tcagtttcat agtgtttcgg tggtcatagc gttagggaaa cgcccgg 47

<210> 518
<211> 53
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Streptomyces

<400> 518
ttcgctagaa cccgataggg tttcggtggt cattgcgtta gggaaacgcc cgg 53

<210> 519
<211> 47
<212> DNA
<213> Flavobacterium resinovorum

<400> 519
gctgcaaccc ctcatgcctg gtgaccatag cgagctggaa ccacccc 47

<210> 520
<211> 52
<212> DNA
<213> Spingobacterium multivorans

<400> 520
taagacagac caataaagat ttttaggtgc ctatatcggc ggtgtctacc tc 52

<210> 521
<211> 53
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechococcus

<400> 521

ccatagagtc acacccttcc tggtgtctat ggcggtatgg aaccactctg acc 53

<210> 522
<211> 60
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived
from species of the genus Synechocystis

<400> 522
agcaaaaccc aaaaatcttt cttggtgtct ttagcgtcat ggaaccactc cgatcccatc 60

<210> 523
<211> 53
<212> DNA
<213> Borrelia burgdorferi

<400> 523
ttttgtcttc cttgtaaaaa ccctggtggt taaagaaaag aggaaacacc tgt 53

<210> 524
<211> 51
<212> DNA
<213> Chlamydia trachomatis

<400> 524
gagaaacgat gccaggatta gcttggtgat aatagagaga gggaaacacc t 51

<210> 525
<211> 138
<212> DNA
<213> Sphingomonas paucimobilis

<400> 525
ctataacctt ggtagtccaa ggtcgagtac aactgctcga tacaagctac aacccaacaa 60
tacttcttcc agattcatgg ccacgctgaa caaagcgtag ggtgggcggc tgtnccgccc 120
acgcgtaact caagcgta 138

<210> 526
<211> 107
<212> DNA
<213> Zymomonas mobilis

<400> 526
ttttgagaac tccactgtca atgtcagcat tgctgacctg ataatgtttt ctcttagctc 60
ttttgaatat cttcgatttt caattaactt cacgcacagg tgtcata 107

<210> 527
<211> 167
<212> DNA
<213> Artificial sequence

<220>
<223> Description of the artificial sequence: derived

from species of the genus Alcaligenes

<400> 527
atacaacacc caagcagttg tatataaagc atcaatcgat tcattaatat gcaaagcaac 60
ttgatttagt tatacgctta gctaaaatga acaaaatata gtaagactca atcagcccat 120
ctgtaaagat ttggaaaacg catcggcaac caataagacc aatgcaa 167

<210> 528
<211> 225
<212> DNA
<213> Borrelia burgdorferi

<400> 528
ctgcgagttc gcgggagagt aagttattgc cagggttttt tatttttttt tagtttttat 60
gttatttaaa tggcttattc aaacaacata aaaaagaaaa tagatattga catggattaa 120
acaaaagata tatattattc tatgttgcat aaacaaattg gcaaagtaga gatggaagat 180
aaaaatatgg tcaaagtaat aagagtctat ggtgaatgcc tagga 225

<210> 529
<211> 681
<212> DNA
<213> Xanthomonas campestris

<400> 529
tggagcaaga cgtcattcgt cctagtcggg cgtcctcaca aattacctgc attcagagat 60
tcataccggc acaggtcggt atgcgaagtc ccttttgggg ccttagctca gctgggagag 120
cacctgcttt gcaagcaggg ggtcgtcggt tcgatcccga caggctccac catattgagt 180
gaaaagactt cgggtctgta gctcaggtgg ttagagcgca cccctgataa gggtgaggtc 240
ggtagttcga gtctacccag acccaccact ctgaatgtag tgcacactta agaatttata 300
tggatcagcg ttgaggctga gacatgttct tttataactt gtgacgtagc gagcgtttga 360
gatatctatc taaacgtgtc gttgaagcta aggcggggac ttcgagtccc taaataattg 420
agtcgtatgt tcgcgttggg tggctttgtt acccacacaa cacgtacatg ttagctccga 480
ggcaacttgg ggttatatgg tcaagcgaat aagcgcacac ggtggatgcc taggcggtca 540
gtggcgatgt aggacgtggt agcctgcgaa aagtgtcggg gagctggcaa caagctttga 600
tccggcaata tccgaatggg gaaacccact gcttcggcag tatcttgcag tgaattcata 660
gctgcttgaa gcgaaccccg t 681

<210> 530
<211> 229
<212> DNA
<213> Cowduria ruminantium

<400> 530
ggtgtgtaag tatggtaaca tatgtagcta accagtacta atagcccgat tgatttactt 60
aatttgtaat tatatgtagt attaaaactg cagcttgtct ttttgcttat tttgttttat 120
agtttaattg ggttggtggt aatagcagaa gtgatacacc cagctacatt tcgaacctgg 180
aagttaagcc ttctagcgct tatggtactt tgtcttaagg cacgggaga 229